



**I Simpósio Internacional de Sanidade Bovina**

# **ANAIS**

**3 a 5 de Novembro de 2022**

**São Paulo**



SIMPÓSIO  
INTERNACIONAL DE  
SANIDADE BOVINA  
xx SANIBOV 2022 xx

# ANAIS DO SIMPÓSIO INTERNACIONAL DE SANIDADE BOVINA

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São Paulo

2022

## Ficha Catalográfica

Elaborada por Rita de Cassia Ortega Borges

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G612 Gomes, Viviani.

Anais do I Simpósio Internacional de Sanidade Bovina / coordenação Alice Maria Melville Paiva Della Libera ; organizadores Viviani Gomes ... [et al.] ; revisado por Adelaide Borges. -- São Paulo, 2022.  
455p.

Trabalhos apresentados no Simpósio realizado nos dias de 3 a 5 de novembro de 2022, por videoconferência e presencial, São Paulo, SP.

Acesso por tempo limitado <https://sanibov.com.br/>

ISBN: 978-65-00-56645-1

1. Bovinos. 2. Complexo respiratório bovino. 3. Ruminantes. 4. Medicina bovina. 5. Doenças do gado bovino. I. Título. II. Alice Maria Melville Paiva Della Libera. III. José Augusto Ferronato. IV. Larissa Padilha. V. Mariluce Cardoso Oliveira.

CRB 8/4852

CDD 636.2



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# ANAIS DO SIMPÓSIO INTERNACIONAL DE SANIDADE BOVINA

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## APRESENTAÇÃO DO EVENTO

O I Simpósio Internacional de Sanidade Bovina – versão 2022 foi preparado em formato híbrido (modalidades presencial e virtual), com participantes de diferentes estados e países. O objetivo geral do SANIBOV é atender o tema SANIDADE BOVINA.

Na versão 2022, o evento abordará o tema “Complexo Respiratório Bovino (CRB)”, cuja programação foi composta por oito palestras teóricas, sessão para apresentação de resumos científicos, mesas-redondas e minicurso prático pós-evento sobre ultrassonografia torácica.

O objetivo geral do SANIBOV 2022 contempla a verticalização do tema “Complexo Respiratório Bovino”, com a interação entre pesquisadores nacionais e internacionais de renome na área, e técnicos que atuam em sistemas de produção de leite e corte no Brasil, Estados Unidos, Canadá e outros países da América Latina.

Atualizar os conhecimentos com a apresentação e discussão de resultados recentes das pesquisas científicas. Pretende-se expandir as fronteiras do conhecimento relativas aos métodos de diagnóstico precoce, aplicação da ultrassonografia torácica, método não-invasivo de diagnóstico de broncopneumonias, o que evita o uso indiscriminado de antimicrobianos por erros diagnósticos, além do estabelecimento do desafio microbiológico, e desenvolvimento de protocolos terapêuticos e emprego das biotecnologias (vacinas nasais e injetáveis) nos programas de prevenção do CRB. Espera-se que o evento seja um meio para transferir os conhecimentos gerados nas universidades para os técnicos e produtores.

Este ANAIS apresenta resumos expandidos das palestras e os resumos científicos para registrar e memorizar este evento tão especial.

Prof<sup>a</sup>. **Viviani Gomes**  
Coordenadora do Evento

Prof<sup>a</sup>. **Alice Maria Melville Paiva Della Libera**  
Coordenadora da Comissão Científica

# PROGRAMAÇÃO



## SCHEDULE

NOV 3-5

NOV 3

2 p.m - Opening - FMVZ/USP Authorities  
02:50 p.m: Clinical examination of the respiratory system: detection of upper (rhinotracheitis) and lower (bronchopneumonia) inflammation, *Prof Dr Roberto Calderon, UNESP Botucatu*  
03:40 p.m: Space Virbac Company  
04:00 p.m: Milk break  
04:20 p.m: Use of field chest ultrasound to detect bronchopneumonia, *Profa Dra. Theresa Ollivett, Madison School of Veterinary Medicine, University of Wisconsin*  
05:10 p.m: Round table, *Profa Dra Alice Maria Melville Paiva Della Libera, FMVZ Usp*  
18h20 às 20h: Poster session

NOV 4

08:00 a.m: Clinical scores and accuracy of diagnostic methods for detecting bronchopneumonia, *Prof Dr Sébastien Buczinski, Professeur titulaire, Université de Montréal*  
08:50 a.m: Microbiological diagnosis of Bovine Respiratory Disease, *Profa. Dra. Amelia R. Woolums, Mississippi State University*  
09:40 a.m: Milk break  
10:00 a.m: Space Elanco Company  
10:20 a.m: Antimicrobial therapy for bronchopneumonia - how to prevent failures in therapeutic responses? *Prof Dra. Theresa Ollivett, Madison School of Veterinary Medicine, University of Wisconsin*  
11:10 a.m: Round Table, *Prof. Enrico Lippi Ortolani, FMVZ Usp*  
02:00 p.m: Vaccination protocols against the Bovine Respiratory Complex - inactivated vs live modified antigens, intranasal vs parenteral, *Prof Dr. Christopher Chase, South Dakota State University*  
02:50 p.m: Vaccinate calves with maternal antibodies: is it effective? *Profa. Dra. Amelia R. Woolums, Mississippi State University*  
03:40 p.m: Milk break  
04:00 p.m: Space MSD Company  
04:20 p.m: Control program against the bovine viral diarrhea virus in dairy production systems in Brazil, *Profa Dra Viviani Gomes, FMVZ Usp*  
05:10 p.m: Round Table, *Prof Dr Paulo Henrique Cunha, UFG*  
06:00 p.m: Awards for the best scientific abstracts

NOV 5

08:00 a.m - 02:00 p.m: Practical course on chest ultrasound  
*Prof. Dr. Sébastien Buczinski, Faculté de Médecine Vétérinaire, Université de Montréal*  
*Prof Dra. Theresa Ollivett, UW-Madison School of Veterinary Medicine*  
*Profa. Dra. Amelia R. Woolums, Mississippi State University*  
Location: AUDAX Farm, Piracicaba - SP



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# PROGRAMAÇÃO

3 - 5 NOV

3 NOV

14h - Abertura - Autoridades FMVZ/USP  
14h50: Exame clínico do aparelho respiratório: detecção de inflamação superior (rinotraqueíte) e inferior (broncopneumonia), Prof Dr Roberto Calderon, UNESP Botucatu  
15h40: Espaço Empresa Virbac  
16h: Milk break  
16h20: Uso da ultrassonografia torácica à campo para detecção de broncopneumonia, Profa Dra. Theresa Ollivett, Madison School of Veterinary Medicine, University of Wisconsin  
17h10: Mesa redonda, Profa Dra Alice Maria Melville Paiva Della Libera, FMVZ Usp  
18h20 às 20h: Sessão dos pôsteres

4 NOV

08h: Escores clínicos e acurácia dos métodos diagnósticos para detecção de broncopneumonias, Prof Dr Sébastien Buczinski, Professeur titulaire, Université de Montréal  
08h50: Diagnóstico microbiológico da Doença respiratória Bovina, Profa. Dra. Amelia R. Woolums, Mississippi State University  
09h40: Milk break  
10h: Espaço empresa - Elanco  
10h20: Terapia antimicrobiana das broncopneumonias - como prevenir falhas nas respostas terapêuticas? Prof Dra. Theresa Ollivett, Madison School of Veterinary Medicine, University of Wisconsin  
11h10: Mesa redonda, Prof. Enrico Lippi Ortolani, FMVZ Usp  
14h: Protocolos de vacinação contra o Complexo Respiratório Bovino - antígenos inativados vs vivos modificados, intranasal vs parenteral, Prof Dr. Christopher Chase, South Dakota State University  
14h50: Vacinar bezerros com anticorpos maternos: é eficaz? Profa. Dra. Amelia R. Woolums, Mississippi State University  
15h40: Milk break  
16h: Espaço empresa - MSD  
16h20: Programa de controle contra o vírus da diarreia viral bovina em sistemas de produção leiteira no Brasil, Profa Dra Viviani Gomes, FMVZ Usp  
17h10: Mesa redonda, Prof Dr Paulo Henrique Cunha, UFG  
18h: Premiação dos melhores resumos científicos

5 NOV

08h - 14h: Curso prático ultrassonografia torácica  
Prof. Dr. Sébastien Buczinski, Faculté de Médecine Vétérinaire, Université de Montréal  
Prof Dra. Theresa Ollivett, UW-Madison School of Veterinary Medicine  
Profa. Dra. Amelia R. Woolums, Mississippi State University  
Local: Fazenda AUDAX, Piracicaba - SP



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## PATROCINADORES



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PALESTRAS



## **ANTIMICROBIAL THERAPY SUCCESS AND HOW PREVENT TREATMENT FAILURES**

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In young cattle, respiratory disease is treated primarily by the administration of long acting, injectable antimicrobials intended for single dose administration. Interestingly, less than 60% of dairy producers consult their veterinarian for specific details about antibiotic usage and 85% use antibiotics in an extra label manner (USDA, 2018). High levels of disease, potential misuse, and retreatment rates contribute to the overall volume of antibiotics administered to dairy calves. This is costly and contributes to the selection pressure for antimicrobial resistance in both pathogenic and commensal bacteria. This is especially concerning because the three classes of antibiotics known for promoting antimicrobial resistance by selecting for multi-drug resistant bacteria in animals and people (3<sup>rd</sup> generation cephalosporins, fluoroquinolones, and macrolides; Guardabassi et al., 2017) are administered to nearly 50% of calves treated for respiratory disease (USDA 2018).

In the field setting, treatment response is either not measured at all, or is assessed indirectly at the herd level by looking at producer treatment records (e.g. retreatment rate, average number of treatments per calf). In the research setting, response is often gauged by clinical cure rate, clinical relapse rate, mortality rate, average daily gain, and severity of lung lesions at necropsy. Using



criteria based largely on resolution of clinical signs, most reports suggest that 20 - 35% of treated calves require multiple antibiotic treatments for relapse or recurrence of their respiratory disease (van Donkersgoed et al., 1993; Windeyer et al., 2012; Heins et al., 2014).

In dairy animals less than 6 months of age, lung ultrasound can rapidly and easily detect the non-aerated or consolidated lung lesions associated with bacterial pneumonia (Ollivett, Buczinski 2016). Depending on the study and regardless of the clinical state of the calf, the sensitivity and specificity of lung ultrasound ranges from 79 – 94% and 94 – 100%, respectively (Rabeling et al., 1994; Buczinski et al., 2015; Ollivett et al., 2015). In addition, there is a high correlation ( $r = 0.92$ ) between the amount of consolidated lung identified on lung ultrasound and gross post-mortem examination (Ollivett et al., 2013) which means we can use this tool to measure the severity of pneumonia in the live calf. Ultrasonographic lung lesions in dairy calves are associated with reduced preweaning ADG (Ollivett, 2014; Cramer et al., 2019), increased mortality (Buczinski et al., 2014), and less milk production during the first lactation (Dunn et al., 2018).

Three BRD subtypes (Ollivett, Buczinski, 2016; Cramer et al., 2019) can be defined when a systematic clinical scoring system, such as the Wisconsin Respiratory Score (McGuirk, Peek, 2014) is incorporated alongside lung ultrasound: 1) upper respiratory tract infections, 2) clinical pneumonia, and 3) subclinical pneumonia. Although the distributions of BRD subtypes will vary from





farm to farm, we have found that at least 1/3 of new cases are subclinical and that for every case of existing clinical respiratory disease, we can expect to find 2 – 4 cases of subclinical disease (Ollivett, Buczinski, 2016; Binversie et al., 2020).

For these reasons, lung ultrasound combined with clinical respiratory scoring has become the primary way that we monitor presence of disease, competency of farm staff for detecting sick calves, and treatment response on local commercial dairies as well as research projects (Ollivett, Buczinski, 2016; Holschbach et al., 2019; Binversie et al., 2020). In regard to measuring treatment response, once treatment has been initiated, the numbers of live bacteria within the lung are significantly reduced and the draw for new neutrophils into the airway slows down. Neutrophils within the airways will undergo apoptosis within 1 - 2 days of arrival and that fibrin and cellular debris will be expelled from the airway through coughing and other cellular mechanisms within 7 – 10 days (Caswell, 2016). This phenomena can be observed ultrasonographically through sequential examinations and lung lesion regression visualized as the airways become aerated again (Holschbach et al., 2019; Binversie et al., 2020).

Unfortunately, data from recent studies suggest that retreatment rates can be 2 to 3 times higher than those reported in the literature (Binversie et al., 2020), ultrasonographic lung lesions associated with pneumonia initially respond to antibiotic therapy but often recur or worsen shortly after treatment (Holschbach et al., 2019; Binversie et al., 2020), and that antibiotic treatment does not always



result in a bacteriological cure within the lung despite early treatment and resolution of clinical disease (Holschbach et al., 2019).

More specifically, the common definition for treatment success (rectal temperature < 104°F, normal respiratory pattern, normal attitude; as reviewed by DeDonder and Apley (DeDonder, Apley, 2015) used by many manufacturers when establishing efficacy of an antibiotic product, would incorrectly classify 100% of the calves with severe lung disease five days after a *Mannheimia haemolytica* challenge study and 14 days after a *Pasteurella multocida* challenge (Ollivett et al., 2013; Holschbach et al., 2019). These findings indicate that despite early recognition of disease, and judicious antibiotic use, bacterial infection has not resolved at the lung level using on-label treatment regimens. We hypothesize that incomplete bacterial killing sets the stage for bacterial replication and relapse or recurrence of consolidation once the antibiotic pressure has been removed. Poor treatment response coupled with misleading clinical criteria for treatment success puts calves at risk for future clinical disease (Binversie et al., 2020) and prolonged periods of slow growth (Cramer et al., 2019).

In summary, individual and herd level factors may contribute to treatment failures and ultrasound guided treatment protocols could re-shape how we measure response to treatment, how we validate dosage regimens for currently approved antimicrobial drugs as well as those drugs undergoing the approval process. Implementing ultrasound guided treatment protocols on farm should improve calf level response, result in fewer relapses, decrease duration of disease, thereby



improving calf welfare and decreasing cost of disease, ameliorate effect of disease, and ensure that administered antibiotics are effective at establishing a bacteriological cure within the lungs.

### #WeanClean™ Philosophy

#### Guiding Principles:

The guiding principle of #WeanClean™ is that calves with healthy, ultrasonographically clean lungs will maintain growth during weaning and will be less likely to require antibiotics for clinical respiratory disease following weaning.

A 6-point scoring system and scoring algorithm is suggested for scoring lung lesions and has served as a practical means to document and monitor lung lesions on commercial dairy farms. To properly score, the operator must be able to recognize the difference between aerated lung, aerated lung with diffuse pleural roughening (also called comet-tail artifacts), lobular lung lesions (also called lobular consolidations or lobular pneumonia), and lobar lung lesions (also called lobar consolidations or lobar pneumonia). In the context of this US scoring system, lobular and lobar lesions simply reflect the extent of which the lung lobe is consolidated on the ultrasound image. Lobular lesions are relatively small discreet areas of consolidation within an otherwise aerated lung lobe. In other words, the hyperechoic pleural interface with reverberation artifact of normal lung can be seen both dorsal and ventral to the lobular lesion when the probe is placed vertically within the rib space. Lobar lesions indicate full thickness consolidation



of the lung lobe that extends proximally from the tip of the lobe. In the US image, the hypoechoic parenchyma of the entire distal lung lobe is visible, and aerated lung cannot be seen ventral to the lesion.

To promote #WeanClean™, use this 4-point ultrasound strategy to measure lung disease at weaning, determine detection and treatment efficiencies, and identify high risk age-groups for follow-up management.

1. Start of weaning – how many have pneumonia at the start of weaning? Goal: < 15%
2. Start of treatment – how many score > 3 or < 2 at their first treatment? Goal: < 15%
3. 7-10 d after treatment – how many score > 2 after their first treatment? Goal: < 15%
4. 12x7 scans – starting at 7d of age, scan 12 at 7d intervals to find high-risk age group

There are only a few general reasons for missing these goals. Understanding these reasons provides a framework for troubleshooting respiratory disease as well as many of the other significant causes of poor health and welfare in young dairy cattle.



- Too many calves weaning with dirty lungs? 3 reasons - they weren't treated, weren't treated right, or poor innate immunity delays response to treatment
- Too many calves with normal or excessively high lung scores at first treatment? 3 reasons – non-respiratory conditions are manifesting as respiratory disease (e.g. sepsis, toxemia, acidosis), we don't spend enough time physically looking at the right calves, or we fail to recognize early signs of clinical respiratory disease
- Too many calves with high lung scores after first treatment? 3 reasons – we used right drug in wrong way (late, wrong dose, duration, frequency), used wrong drug (wrong class, resistant bug), or poor innate immunity delays response
- Who are the high-risk calves and does age at first treatment reflect this reality? Use 12x7 scans to confirm onset of disease, train treaters to focus on the right calves, and develop an early detection and treatment program.

Weaning is stressful. Calves need to cope not only with changes to their diet, but to changes in housing, social interactions, and processing interventions such as vaccinations. Failing to prevent and adequately cure respiratory disease results in a large population of calves that must now undergo this already stressful time with chronic, typically subclinical, pneumonia which affects performance and is a



welfare issue. As caretakers, veterinarians, and consultants, we need to make sure that calves wean with clean lungs.

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## **CALF LUNG ULTRASOUND NOTES WITH ALGORITHM AND SCORING**

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Respiratory disease is economically challenging, subclinical disease exists, and producer-based diagnoses lacks in sensitivity. Therefore, monitoring lung health in young cattle should be considered a priority for maintaining proper drug use, animal well-being, and profitability. Incorporating calf lung ultrasound at the strategic points mentioned above will not only provide in understanding of the epidemiology of respiratory disease in client herds but more importantly will help identify high risk age groups and treatment failure problems before they become catastrophic.

### **Ultrasound Equipment, Calf Preparation, Restraint, Technique**

Transrectal probes permit the best access to the axillary region and cranial thorax of the young dairy calf. They are widely used by bovine veterinarians making them most suitable for practical field-based lung ultrasound in young cattle. You can choose between using a machine with an attached screen, a wireless screen, or goggles, based on personal preferences.

Operator positioning, restraint, and transducing agents are all necessary considerations when scanning. During the examination, it is up to the operator to



decide whether to stand or squat down next to the calf. Operator position is often dependent on whether an assistant is available to help restrain. In most situations, restraint should be minimal, rarely requiring a halter, headlock, or chute, particularly in young preweaned dairy animals. Increasing the level of restraint often only manages to increase handling time, therefore reducing the practicality of the procedure.

In preweaning dairy calves, I find it easiest to do both the restraining and scanning myself as I often am working alone. To simultaneously restrain and scan, place the calf's rear-end in the corner of the pen, face the calf, and straddle her neck with your legs closed together in front of her shoulders. This is like straddling the calf to collect jugular blood, just facing the opposite direction. Working alone, in this manner, I expect to scan 30-45 calves/hour. Veterinarians that use an assistant to provide restraint and document findings can easily scan 75 calves/hour.

On the farm, 70% isopropyl alcohol is the transducing agent of choice, and the hair is not clipped or shaved from the chest. 90% (or greater) isopropyl alcohol is recommended for use when scanning calves in arid climates. Coupling gel, vegetable oil, and gel/liquid alcohol-based hand sanitizer will also work if you find conventional isopropyl alcohol difficult to obtain as many of us experienced early in the COVID-19 pandemic. Significantly less alcohol is required if a household spritzer/spray bottle or a small garden sprayer is used.

Technique is critical. Within reason, the ultrasound technique can be modified based on the goals of the exam. Individual sick or "poor-doing" animals are more likely than the average calf to harbor lesions, specifically lung abscesses, in the



caudal lung lobe. In these cases, the caudal lung lobe should always be assessed as well as the more cranial lung lobes. This requires scanning the right lung from the 10<sup>th</sup> intercostal space (ICS) cranial to the 1<sup>st</sup> ICS and the left lung from the 10<sup>th</sup> ICS cranial to the 2<sup>nd</sup> ICS.

When screening a group of calves for pneumonia, a different approach can be taken compared to that used for an individual sick animal. In this situation, lung ultrasound is used to screen specifically for bronchopneumonia (most common finding) and interstitial disease. Bronchopneumonia reliably localizes to three specific lung lobes particularly during the early phase of disease. The cranial aspect of the right cranial lung lobe is most commonly affected, followed by the caudal aspect and right middle lung lobe, then the caudal aspect of the left cranial lung lobe. The cranial aspect of the left cranial lobe and the caudal lung lobes are rarely consolidated without consolidation of the previously mentioned lobes.

Studies that have been designed to detect the onset of new cases of BRD such as Binversie (2020), Cramer (2019), and Holschbach (2019), demonstrate that subclinical pneumonia comprises at least 50 - 90% of new cases of BRD in both naturally occurring disease in commercial herds as well as in experimentally induced pneumonia in the laboratory setting. In addition, Binversie (2020) and Cramer (2019) also show that up to 30% of new cases can be upper respiratory disease (CRS+ with 2 categories scoring 2 or higher and US < 1cm). Since lung ultrasound will fail to detect clinical respiratory disease not due to bacterial bronchopneumonia or interstitial pneumonia, this latter fact is the rationale behind using a clinical scoring system such as the UW score (CRS) as one component



of identifying diseased calves. As such, both CRS and US must be used to define positive; however, they should not be combined into a single definition (e.g. BRD+ = both CRS and US are abnormal). Instead, at least two independent definitions for BRD+ should be used concurrently: definition 1) CRS+ (with or without lung consolidation) and definition 2)  $\geq 1$  cm or more lung consolidation.

The key to accurate lung ultrasound is being systematic. A systematic approach depends on an understanding of the external thoracic anatomy of the calf, the internal anatomy of the lung, and appropriate ultrasonographic landmarks. The external anatomy of the calf refers to the specific intercostal space where the probe is placed. The internal anatomy refers to the specific lung lobes that are being evaluated. Lastly, the ventral image landmarks provide unique identifiers for each lung to ensure that the high-risk locations for pneumonia are examined. Once comfortable with the technique and scoring system, an accurate ultrasonographic diagnosis can be made within 20-30 seconds. Landmarks are outlined in the attached document.

In general, the recommended lung ultrasound examination extends from the caudal thorax to the cranial thorax by moving the probe along the grain of the hair in a dorsal to ventral fashion within each ICS. The probe should move parallel to the rib within the ICS. It is a common mistake to move the probe perpendicular to the ground. Instead, the probe should be moved slightly caudally staying within one ICS to avoid imaging the rib. Very slight adjustments can move the ultrasound beam onto or off the rib surface and/or enhance visualization of a lung lesion. These small movements include moving the tip (or the end) of the probe side to side or rotating the footprint (the portion of the probe



in contact with the body wall) so that it is facing more cranial or caudal within the ICS. If the rib obscures the image of the lung, simply stop moving, readjust the angle of the probe until the lung is present, and then continue ventrally within the ICS.

The attached 6-point scoring system and scoring algorithm is suggested for scoring lung lesions and has served as a practical means to document and monitor lung lesions on commercial dairy farms.

In order to properly score, the operator must be able to recognize the difference between aerated lung, aerated lung with diffuse pleural roughening (also called comet-tail artifacts), lobular lung lesions (also called lobular consolidations or lobular pneumonia), and lobar lung lesions (also called lobar consolidations or lobar pneumonia). In the context of this US scoring system, lobular and lobar lesions simply reflect the extent of which the lung lobe is consolidated on the ultrasound image. Lobular lesions are relatively small discreet areas of consolidation within an otherwise aerated lung lobe. In other words, the hyperechoic pleural interface with reverberation artifact of normal lung can be seen both dorsal and ventral to the lobular lesion when the probe is placed vertically within the rib space. Lobar lesions indicate full thickness consolidation of the lung lobe that extends proximally from the tip of the lobe. In the US image, the hypoechoic parenchyma of the entire distal lung lobe is visible, and aerated lung cannot be seen ventral to the lesion.

In general, US score 1 is considered interstitial disease (heavy, wet and rubbery lungs associated with septicemia, viral infection, or pulmonary edema) and ultrasound scores  $\geq 2$  are consistent with bacterial bronchopneumonia (Ollivett



TL, 2015). Abnormalities such as pneumothorax, pleural fluid, abscesses, and necrosis are not inherently included in the scoring system. Instead, a comment is included within the record regarding the abnormality (e.g. US score 4 plus 4 cm abscess in right caudal lung lobe at the level of the 8<sup>th</sup> intercostal space).

## Summary

The portable rectal ultrasound machines already in use by bovine veterinarians for reproductive examinations are a fast, accurate, and practical means of diagnosing the lung lesions associated with BRD in young cattle. When combined with respiratory scoring, systematic calf lung ultrasound allows for the differentiation of BRD into specific practical subtypes including upper respiratory tract disease, clinical pneumonia, and subclinical pneumonia; all of which can be performance limiting. In individuals, lung ultrasound can be used to identify poor prognostic indicators such as caudal lung lobe consolidation, lung abscesses, and lung necrosis, and can aid culling and purchasing decisions. At the herd level, lung ultrasound can be used to identify specific populations at risk for developing the subtypes of BRD, monitor the prevalence and severity of BRD over time, and evaluate the impacts of management changes such as ventilation, vaccination, changes in treatment protocols or personnel. In conclusion, calf lung ultrasound can add to the services provided by bovine veterinarians, increasing their value and impact on animal health.



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## Algorithm for scoring lung lesions in dairy calves

1. Understand the orientation of your probe
  2. Identify ventral image landmarks
  3. Determine if lung is aerated or non-aerated
  4. If lung is non-aerated, determine if the lesion represents lobular or lobar pneumonia
  5. If lobar pneumonia is identified, count the number of lobes affected to create the ultrasound score.
- 

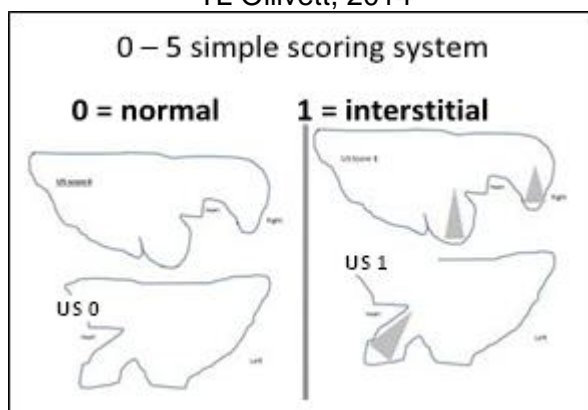
- 1. Understand the orientation of your probe**
  - a. Touch your finger to the tip of probe which will be dorsal in the intercostal space
  - b. Ollivett images are always in the same orientation
    - i. Left side of image = Dorsal
    - ii. Right side of image = Ventral
- 2. Identify the ventral image landmark in your image**
  - a. What landmark do you see?
    - i. Diaphragm?
    - ii. Costochondral junction and pleural deviation?
    - iii. Heart?
    - iv. Internal thoracic artery and vein?
    - v. Liver?
    - vi. Kidney?
- 3. Determine if lung is aerated or non-aerated**
  - a. Is reverberation artifact present?
    - i. Continuous?
      1. Aerated (normal) lung
        - a. Few to no comet tails- US Score 0
        - b. Severe, diffuse comet-tailing – US Score 1
    - ii. Interrupted?
      1. Non-aerated (typically consolidated) lung



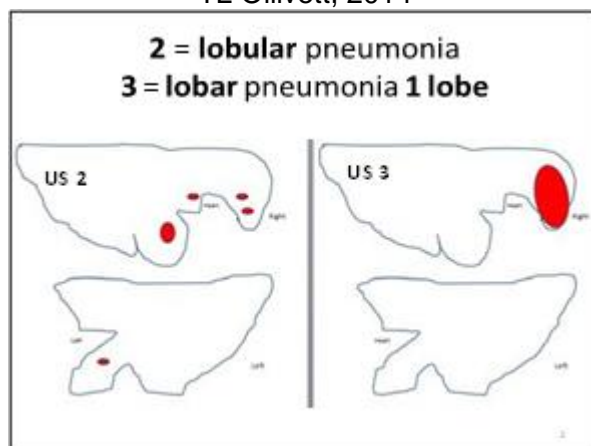
4. **If lung is non-aerated, determine if the lesion represents lobular or lobar pneumonia**
  - a. Is there a gap present in the reverberation artifact?
    - i. Yes- lobular lesion – US Score 2
  - b. Does the reverberation artifact and pleural line terminate prematurely resulting in the hypoechoic architecture of the lung lobe being visible?
    - i. Yes – lobar lesion
5. **If lobar pneumonia is identified, count the number of lobes affected to create the US Score.**
  - a. US Score 3: 1 lobe
  - b. US Score 4: 2 lobes
  - c. US Score 5: 3 or more lobe

### Lung Lesion Scoring Chart for Thoracic Ultrasonography of Dairy Calves

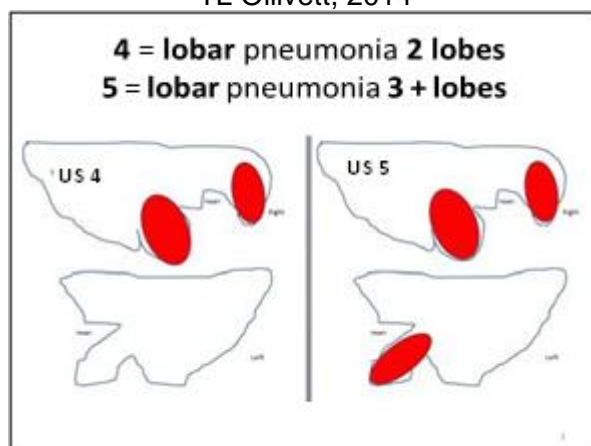
TL Ollivett, 2014



TL Olivett, 2014



TL Olivett, 2014





## CLINICAL EXAMINATION OF THE RESPIRATORY SYSTEM OF CATTLE

Prof. Dr. **Roberto Calderon Gonçalves** e Dr<sup>a</sup>. **Bianca Paola Santarosa**

Diseases of the respiratory system in cattle are considered a serious animal health problem, as they lead to high rates of morbidity and mortality, in addition to economic losses resulting from low feed conversion, delay in weight gain, decrease in productivity, costs with diagnosis and treatment. The early diagnosis of a disease is of great importance for the institution of treatment and prevention of new episodes of disease, not only in the examined animal, but in the herd as a whole.

The diseases are manifested by clinical signs identified in the semiological examination, and must be interpreted correctly. Thus, for an adequate diagnostic procedure, it is necessary to carry out a good clinical examination, since no auxiliary test can effectively replace it. Despite this, it is often necessary to use complementary exams for the differential diagnosis of respiratory diseases.

It is necessary to locate the process within the respiratory system, whether it is restricted to the anterior or posterior airways, and whether it includes the pulmonary interstitium. The anterior airways include the nostrils, sinuses, pharynx, larynx, and trachea. The greater bronchi, segmental bronchi and bronchioles belong to the posterior airways, which carry inhaled air to the alveolar sacs and alveoli for gas exchange (hematosis). Through the semiological examination, considering the history or anamnesis, inspection, palpation, percussion, auscultation and olfaction, the clinician is able to locate the pathology, if possible, establish its nature and etiology.



## ANAMNESIS

Focusing on the respiratory system, it is important to extract from history whether the problem is individual or collective. Diseases in a single individual can be related only to him, but they can also be the beginning of a process that affects the herd. Thus, there is always a need to pay attention to the emergence of new cases. If the problem is a collective one, what should be obtained from history is how much it is affecting the herd, with the calculation of morbidity and lethality rates.

Other important points are information about the beginning of the process, whether it was in the form of an outbreak, or sporadic cases occur over time. The time and type of evolution must be taken into account, in order to verify the severity of the case, the pathogenicity and transmission of the offending agent. Treatments prior to attendance should be explored, for eventual changes in the therapeutic plan to be adopted.

Within respiratory problems, there is always a need to collect all the clinical symptoms observed, and to establish a close relationship between the clinical signs presented and the moment when they occur with greater intensity during the handling of the animal. As clinical manifestations of diseases of the respiratory system that can be perceived by the tutor, there are: runny nose, sneezing, coughing, fatigue during exercise, noises heard during breathing, rapid and shallow breathing (tachypnea) and respiratory difficulty (dyspnea).

Cough may be exacerbated or present only during feeding and, at this



time, information must be collected and relationships established between the type of feed, whether powdery or not, and the height of the feeding trough, which can cause constant trauma. in the trachea or larynx. Dry and constant coughs, especially during exercise, are usually related to tracheal or tracheobronchial inflammatory problems.

The runny nose can be more intense when the animal lowers its head to eat. This is not always related to the effect of food on nasal secretion, but rather to the physical effect of facilitating its drainage to the outside. However, it can be one of the first signs of alteration of the respiratory system, whether in cases of rhinitis or increased secretion in the respiratory tree.

It is extremely important to describe the facilities, where humidity, internal temperature, ventilation and insolation must be observed, and the type of bedding used, whether it is powdery or has irritating agents, such as straw or moldy hay. All of these are predisposing factors for respiratory infections, especially in younger animals.



## **SEMIOLOGICAL METHODS**

### **INSPECTION**

Inspection is the semiological method in which the animal as a whole is observed and, in this case particularly, the respiratory system. The animal should be observed preferably without touching it and without exciting it, as this may cause changes in respiratory rate and even in the type of breathing. In large animals, the examiner should look at it obliquely, preferably at the back or front of the animal, in such a way that the costo-abdominal transition point can be observed. Count the respiratory rate (RR) in one minute (mpm) and check the respiratory type and rhythm. In healthy adult cattle, the FR is 10 to 30mpm, and in young cattle, 24 to 36mpm.

Physiological oscillations in respiratory rate (RR): the lower the age of the animal, the higher the RR, which decreases with advancing age; large animals and obese animals have lower RF than small and thin animals; the RR gradually increases during pregnancy and is higher during exercise, and in hot and humid environments and in stressful situations.

Pathological oscillations in respiratory rate: These are characterized by tachypnea, bradypnea, or apnea. Tachypnea is an increase in RR. Occurs in situations of fever, pain or decreased blood oxygenation; Bradypnea: is the decrease





in RR. It can occur in depressions of the central nervous system or close to the death of the animal; Apnea: is the total absence of breathing.

Another particularity that must be observed during the inspection is the respiratory rhythm. When measuring the RR, it should be observed if the respiratory rhythm is within the patterns considered normal or if variations are occurring, which can help the clinician in the diagnosis of respiratory injury. The normal rhythm is observed as an in-breath, a short pause, an out-breath, and a longer pause, followed by an in-breath. Any change in respiratory rhythm is called a respiratory arrhythmia.

By observing breathing, that is, the relationship between inspiration and expiration, chest and abdomen movements and the posture adopted by the animal, respiratory activity can be classified as normal (eupnea) or difficult (dyspnea). In normal breathing, the inspiratory movement is active and faster than the expiratory movement, which is passive and maintains a temporal ratio of 1: 1.2 respectively.

The classification of dyspnea can be of great help in locating the respiratory process. Inspiratory dyspnea is related to changes in the anterior airways, whether due to stenosis, foreign bodies or inflammation, which reduce the airway lumen, making it difficult for air to enter. This is easily understood, as the anterior airways have little support and, as a consequence, when the animal makes forced inspirations, the tendency of the airways, which are outside the thoracic cavity, is to “collapse” and, therefore, to decrease in their caliber. intraluminal will make it difficult for air to enter the respiratory tree.

Expiratory dyspnea is related to morbid processes that reduce the elasticity of pulmonary return or cause small airway obstructions, making it difficult for air to



escape, as in pulmonary emphysema, bronchitis and bronchiolitis. In this case, we can understand the process if we observe that, during inspiration, there is negative intrathoracic pressure and, therefore, expansion of the airways that are inside the thorax. During expiration, as the negative pressure decreases, there is compression of the chest walls on the lung and, consequently, on the intrathoracic pathways. If there are foreign bodies, excess mucus or any other problem that reduces the lumen of these small pathways, there will be difficulty in leaving the alveolar air and, consequently, greater effort to exhale. In pulmonary edema, dyspnea is of the mixed type, because the lung, due to the presence of fluid in the interstitium, will have difficulty expanding and, consequently, inspiratory dyspnea. With the outflow of fluid from the blood vessels into the bronchioles, there will also be difficulty in the outflow of air from the alveoli, characterizing mixed dyspnea. Despite this, in this disease in particular, there is a predominance of inspiratory dyspnea due to the greater difficulty in gas exchange and the resulting great hypoxia. In bronchopneumonia, dyspnea is of the mixed type, as there is difficulty in lung expansion (inspiratory dyspnea) due to congestion caused by inflammation and exudate outflow in the bronchi and bronchioles, which determines difficulty in expiration (expiratory dyspnea).

The lung can functionally respond to the decrease in gas exchange by two compensatory mechanisms: increasing respiratory rate (tachypnea) and amplitude (hyperpnea). Tachypnea does not help to locate the change within the respiratory system, as small anatomical changes, as in focal pneumonias, already cause changes in gas exchange, stimulating this compensation mechanism. Hyperpnea, in turn, is mainly related to processes that make lung expansion difficult, such as pneumothorax or, temporarily, right after exercise.



The respiratory type is another characteristic that may indicate the location of the pathological change within the respiratory system. The normal respiratory type in domestic animals is the costo-abdominal one, which may change to the costal and abdominal type. Animals with processes that manifest chest pain, such as rib fractures or pleuritis, may present the respiratory type or breathing, predominantly abdominal, as a form of defense against pain. Those who have abdominal pain, such as with peritonitis or severe compressions on the diaphragm (eg, gastric dilatations), may experience costal breathing.

Nasal inspection is of great importance in the examination of the respiratory system. Changes in the nasal mirror, also called muflo, should be observed if there is dryness, such as in cases of fever, dehydration or hypovolemia; erosions, as in malignant bluetongue, or any other types of lesions that may indicate or lead to alterations in breathing. In the canal, also called nasal fossa, changes in the color and moisture of the mucosa should be checked and lesions such as ulcers, erosions, polyps, tumors and foreign bodies must be looked for, which can be seen in the direct inspection of the nasal cavity. The healthy nasal mucosa is pink, moist and has no visible lesions.

In the inspection of the nostrils, nasal discharge may provide information suggestive of the location of the morbid process. Runny nose can come from the anterior or posterior respiratory tract. If it is unilateral, it may indicate changes in the corresponding nostril and the most common processes are foreign bodies, ulcers and local wounds. If it is bilateral, it may represent involvement of both nostrils, especially in inflammatory processes that increase nasal secretion, or it may originate from sites



located posterior to the nostril, such as the larynx, trachea and bronchi, affected by conditions that increase the amount of inflammatory secretions in these airways. Nasal discharge must be analyzed according to its type, being classified as serous, mucous, purulent and hemorrhagic or their combinations.

When examining the nostrils, attention should be paid to the odor of the breath and the flow of exhaled air. Putrid odor of breath is related to lesions where there is tissue destruction, such as in necrotic laryngitis, in lung abscesses, or in aspiration pneumonia. Unequal airflow in the nostrils implies a decrease in the caliber of one of them, which can be found in obstructions by foreign bodies, tumors or any other problems that cause stenosis in the nostril lumen with less airflow. In the same way it is important to feel, with the back of your hands, the temperature of the exhaled air. The increase in temperature in the air flow out of one of the nostrils is indicative of an inflammatory process in the corresponding nasal cavity.

Cough is one of the mechanisms for cleaning the respiratory system and occurs when there is irritation of the nerve endings of the larynx and trachea caused by inflammation of the mucosa, either by the direct action of the aggressive agent on the mucosa or by excessive production of mucus. Dry and constant cough usually indicates inflammatory alteration in the upper airways, as in pharyngitis and laryngitis, and may also be present in tracheitis. Wet or productive cough is related to increased bronchopulmonary exudate, as in bronchopneumonia, as the inflammatory fluid moves in the airways with breathing, stimulating coughing. To confirm the information received from the animal's tutor and to verify the type of cough, the cough reflex



should be performed, which is stimulated by compression and friction movements or by pinching the first tracheal rings.

## **PALPATION**

In addition to the cough reflex described above, which is already a tracheal palpation maneuver, all external parts of the respiratory system should be palpated in search of depressions (sinking of the nasal bone, cervical tracheal ring fracture, rib fracture) or increases in volume, which may or may not have been verified by inspection. In these superficial alterations, the classic signs of inflammation (pain, heat, redness, tumor) are always observed and, depending on the location found, they can be related to localized inflammatory processes, such as, for example, abscesses. If these signs of inflammation are felt in the intercostal spaces, without increases in volume in this region, it is an important indication of pleuritis; if there is pyothorax associated with pleuritis, there is, in addition to an increase in temperature, bulging of the intercostal spaces, seen on inspection in lean animals.

If on palpation, the submandibular lymph nodes are enlarged and there is cough on palpation of the larynx, it may be an indication of inflammation of the throat, characterizing processes such as pharyngitis, laryngitis or abscesses. This examination must therefore be accompanied by an internal inspection of the pharynx, either by direct inspection with mouth openers and tongue depressors or with the aid of endoscopy.



On palpation, vibration may be felt at the level of the larynx or trachea, which is called laryngeal or tracheal thrill and is indicative of excessive fluid or membranes that vibrate with the passage of air. In the same way, thoracic thrill can be felt, which has the clinical significance of the presence of fluid (inflammatory or not) in the bronchi, of pleural friction (pleural rub) or, when felt over the cardiac area, of a heart murmur or pericardial rub.

## **PERCUSSION**

Percussion should be performed from the paranasal sinuses to the posterior portion of the thorax. In the paranasal sinuses (frontal, lacrimal and maxillary) the percussion must be made with the handle of the percussion hammer and in a comparative way between the left and right sides of the animal's face. The main change that can be heard is the change from normal (clear) to massive sound, indicating that a cavity that was previously empty is being filled by some substance, for example, pus. This sign suggests sinusitis or tumors in the paranasal sinus, that is, lesions that occupy space. On the other hand, if there is accumulation of gas, the sound can change to tympanic, as for example, in cases of infection of the sinus cavity by anaerobic bacteria, which produce gas.

The sound response to chest percussion can range from normal (clear) sound to clinically significant sound changes. Areas of sub-massive or massive sounds may indicate that the lung parenchyma is filled with solid tissues or that, at least, decreases the amount of air in the organ, as in cases of pneumonia, abscesses or



lung tumors. Areas of tympanic sound indicate increased air filling of the lung and may be related to pulmonary emphysema or pneumothorax.

Pathological changes must be close to the chest wall and of sufficient size so that they can be perceived by this semiological method, as the sound produced by percussion penetrates 4 to 7 cm into the lung. The technique is performed with the animal standing in a silent environment. It can be digit-digital or hammer-plesymetric. Dorsoventral and craniocaudal should be performed in the entire thoracic area, moving the plesimeter in the intercostal spaces. The anterior limits are musculature of the scapula (massive sound); the superior ones the dorsal musculature (massive sound); and the posterior ones: observing the crossing of lines that pass, imaginary, in the intercostal spaces (ICE) with lines, imaginary and horizontal, that pass over the iliac tuberosity (iliac line, in ruminants 12th IIC), ischial tuberosity (ischial line, 11th EIC) and at the scapulohumeral joint (the meeting line, 8th EIC). The sound in the most central region of the chest is called clear. Advancing backwards, at the posterior limit, it becomes tympanic or submassive (depending on whether there is more or less gas content in the abdominal structures). In the lower chest the sound is submassive.

## **AUSCULTATION**

Auscultation is the diagnostic method that provides more information about the functioning of the respiratory system. The upper airways and the thoracic region should be auscultated separately, although it should not be forgotten that



the auscultation of one area may interfere with the other, especially the sounds produced in the anterior airways, which may interfere with the auscultation of the lungs.

The animal should preferably be stationary and at rest. Auscultation can be performed directly, with the ear on a towel on the chest, or indirectly, with a stethoscope. At least two respiratory movements should be auscultated at each site. For a more accurate diagnosis, the application of light exercise (walking) or the temporary inhibition of the animal's breathing may be necessary, maneuvers that intensify the respiratory sounds. Also, a plastic bag can be fitted on the snout, called a breathing bag, without, however, obliterating the nostrils, but simply increasing the CO<sub>2</sub> content in the inhaled air. The sounds that can be heard are divided into normal sounds, with their pathological variations, and pathological sounds, also called respiratory adventitious sounds.

Normal sounds are produced by the turbulence of airflow in airways greater than 2 mm in diameter, and may vary in quality depending on the location of the stethoscope, the speed of air during breathing, and the amount of tissue over the area being heard.

The laryngotracheal noise is caused by the vibration of the walls of the larynx and trachea, being heard over the region of the cervical trachea, when the air passes through. Pathologically, tracheal stridor can be heard in this region, associated with cases of laryngeal or tracheal stenosis, and tracheal crackles, caused by the accumulation of fluid or mucus in these places, as if it were the bursting of bubbles.





In the thoracic area, tracheobronchial noise is heard, which is the sound produced by the passage of air through the large bronchi and the final portion of the trachea, with vibration of its walls. It is a rude noise, heard in the anterior third of the chest, both during inspiration and expiration.

Another sound heard in the thoracic area is the bronchobronchiolar noise, which is the respiratory noise produced by the vibration of the walls of the smaller bronchi and bronchioles. It is a soft sound, heard in the posterior two-thirds of the chest, during inspiration.

## **VARIATION OF NORMAL RESPIRATORY NOISE**

Basically, the increase in intensity of normal lung sounds on auscultation means an increase in the amount of air that penetrates this organ and, consequently, greater vibration of the walls of the airways. Whenever there is an increase in breathing intensity, either due to an increase in respiratory rate (tachypnea), in amplitude (hyperpnea), or even due to respiratory difficulty (dyspnea), there will be an exacerbation in the auscultation of respiratory sounds.

Normal breath sounds may also be exacerbated in cases where their transmission is facilitated, especially when there is fluid in the lung interstitium, increasing its density. The pathological processes that lead to the deposition of fluid in the pulmonary interstitium, thus increasing sound transmission, are pneumonia, congestion and pulmonary edema.



## Pathological or Adventitious Sounds

Coarse crepitus clinically means an increase in fluid within the bronchi, whether inflammatory or not. The air passing through the bronchi, which have an exaggerated amount of fluid, determines the formation of a wave, sufficient to cause obstruction of its light. As the pressure before and after this liquid wave are different, the tendency is for the bronchial lumen to clear, followed by a new obliteration, as the air continues to enter. It can be detected in cases of bronchopneumonia and pulmonary edema.

Fine Crackling is the sound similar to the rubbing of hair near the ear, or the bursting of small bubbles. This noise is produced during detachment of the walls of the small airways filled with excess fluid or mucus. If it is inspiratory, it can mean pulmonary edema or pneumonia and if it is expiratory or inspiratory/expiratory (mixed), chronic obstructive pulmonary disease, bronchiolitis, and pulmonary emphysema.

Interrupted Inspiration are small interruptions in inspiration. If this noise is heard during inspiration, with the chest wall moving all at once, it is an indication of sequential obstruction of the bronchi, with fluid in insufficient quantity and viscosity to cause coarse crepitus. If noise is heard with the chest moving in two beats, it is suggestive of pain on inspiration (pleuritis) or psychic excitation of the animal.



Wheezing is a high-pitched, high-pitched sound that resembles a hiss or hiss. Indicates airway narrowing, caused by deposition of sticky viscous secretion, which deforms the tubular lumen. If it occurs at the beginning of inspiration, it is mainly related to extrathoracic processes, such as laryngeal stenosis, compression of the trachea, or thick mucus deposited at these sites. If it appears at the end of inspiration or expiration, it may indicate obstruction of the small airways, as in cases of bronchitis or bronchiolitis and chronic obstructive pulmonary disease.

Snoring is a low-pitched, high-intensity sound produced by the vibration of viscous secretions adhered to the walls of large bronchi, during the passage of air. This sound may indicate bronchopneumonia, if its origin, that is, its maximum auscultation point is in the chest, or show laryngitis or laryngotracheitis, if it is better heard in the larynx or trachea region.

Pleural rubbing is the noise caused by the friction of inflamed visceral and parietal pleura, indicating pleuritis. In a healthy animal, the pleurae slide smoothly, one over the other, without making any noise. When there is inflammation and fibrin deposition on them, friction is transmitted to the examiner's ear as if it were the rubbing of two sheets of paper.

Murmur, rub, or Cardiopleural Noise is harsh, similar to the scraping of two rough surfaces, heard during inspiration and coincident with cardiac movement. It corresponds to the friction of the pleura over the inflamed pericardium, thus indicating pleuritis associated with pericarditis.



Murmur or Cardiopulmonary Noise is soft, low-pitched, similar to pursing your lips. The entry of air passing through the smaller bronchi from the lung areas overlying the heart may be interrupted during the isometric contraction period of ventricular systole. When systole occurs, the passage of air is released, and this type of interrupted sound is heard, sequentially with each new beginning of systole. It occurs in healthy animals or in those in which there is excess mucus production, as in bronchiolitis.

Bronchophony is noise propagated from the anterior airways, such as voice, moaning, coughing, crackles or laryngeal stridor. They are heard, sometimes as imprecise buzzing, sometimes clearly, mainly in the anterior region of the thorax.

Accessory noises that disturb auscultation are the noises of cutaneous muscle contractions, hair crackling, swallowing noises and gastroenteric noises. All these sounds and more those produced in the environment can be heard when the respiratory system is auscultated, making the diagnosis difficult.

## **AUXILIARY EXAMS IN THE DIAGNOSIS OF RESPIRATORY DISEASES**

Despite the clinical examination having absolute importance in the diagnosis of respiratory diseases, some complementary tests are extremely important for the diagnosis, such as blood count; feces parasitological examination; serological antibody titration; radiography; chest ultrasound; endoscopy; tracheobronchial and bronchoalveolar lavage; blood gas analysis;



Lung biopsy; thoracentesis (5th EIC on the left side or 4th EIC on the right side); and necropsy. With the increasing use of auxiliary methods, the clinician is acquiring great help for the diagnosis and study of respiratory diseases. Many of the methods are expensive or impractical and are only justified in some animals. Others need more in-depth studies to be used in routine diagnosis. But, without a doubt, future work in this area will increasingly provide means for the rapid and safe diagnosis of respiratory diseases in animals and, with this, minimize the consequent economic losses and decrease in productivity.

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## **CONTROL PROGRAM AGAINST THE BOVINE VIRAL DIARRHEA VIRUS (BVDV) IN DAIRY PRODUCTION SYSTEMS IN BRAZIL**

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Bovine Viral Diarrhea (BVD) is responsible for severe economic losses in both dairy and beef industry. Bovine Viral Diarrhea Virus (BVDV) belongs to the Flaviviridae family, genus Pestivirus, which contain 11 different species, including BVDV-1, BVDV-2 and Hobi-like-Pestivirus (HoBiPev) (ICTV 2020).

Bovine viral diarrhea virus can be transmitted both horizontally and vertically, where vertical transmission in early pregnancy may result in the birth of persistently infected calves. BVDV reproductive infection during a window of 40-125 days of gestation with non-cytopathic (NCP) BVDV generates persistently infected calves (PIs). This is before the fetus develops the capacity to mount an adaptive immune response (antibody). Persistently infected cattle are the most important source for spread of the virus.

PI cattle have a short lifespan, which is often less than one year. However, they can achieve the reproductive and productive age depends on the health management offered by the dairy farm. Our previous publication described the secondary clinical diseases, milk production and quality, and reproduction performance in Holstein heifers and cows from a single large commercial herd persistently infected with BVDV type 2 (BASQUEIRA et al., 2020). The most



frequent disease detected in the PI animals was Bovine Respiratory Disease (BRD), especially in adult cows aged from 25 to 36 months (50%). The PI cows had a 1.615 greater chance of developing BRD than the control group ( $p = 0.012$ , IC 95% = 1.155–2.259). In addition, PI cattle had a three-times greater chance of developing post-partum disease than paired control group (IC 95% = 1.348–6.678).

After birth, BVDV infection can be acquired by different routes of transmission: aerosol, fomite and oral by the contact with PI animals, which results in transitory or acute infection. In this cases, the clinical disease ranges from mild and unapparent infection to severe disease, leading to rapid death, depends on the properties of the infecting strain, and the immune and physiological state of the host. In addition, it is known that BVDV leads to immunosuppression, which enhances the probability that cattle are secondarily infected by other pathogens.

The integrated control program of BVDV depends on three main focuses: 1. identification of positive herds and persistently infection animals (PIs), followed by PIs culling; 2. vaccination of susceptible animals; 3. implementation of biosecurity management practices.

Briefly, the identification of positive herds based on bulk milk tank test. PI screening has conducted in the last generation of calves and heifers, in addition to dairy cattle without live offspring. Ear skin samples (ear notches measuring 1 cm × 0.5 cm) have been obtained from the dorsal pinna margin by using a stainless-steel ear notching clamp (type V pig), stored in a sterile microtube at  $-20\text{ }^{\circ}\text{C}$ . The identification of BVDV have been done by using virus isolation, Erns



antigen specific ELISA test, traditional RT-PCR and Real-time PCR. After 30 days, positive animals in the 1st test should be retested to differentiate transitory and persistently infection. All live dams and grandams of the animals identified as PI among the heifers and calves should be tested. After the removal of all PI animals identified in this herd, all newborn calves should be tested monthly by ear notch sampling at least until 12 months after the last PI identified.

Vaccination is a tool to enhance the immunity of susceptible animals against BVDV. In Brazil, the most vaccines formulation against BVDV are polyvalent and composed by inactivated virus. Although, there is a current trend to the introduction and registration of new products containing modified live virus (MLV) and recombinant vaccines. In general, MLV vaccines are more immunogenic than inactivated ones. The antigens in this presentation have a greater ability to penetrate and multiply in host immune cells, which results in greater intensity and duration of the humoral immune response, in addition to the advantage to require only an annual dose (BACCILI, 2017). On the other hand, there are some concerns regarding the biological risk of the MLV adoption in the field due to the return to virulence of the strains included in the MLV vaccines and possible mutations, especially in BVDV free herds. Inactivated vaccines do not have risk of virus replication, but these products tend to be more expensive due to the higher amount of antigenic mass in its formulations, which generates greater risks of undesirable adverse reactions, and it are less immunogenic and require repeated booster (CHASE, 2007).

There was an expansion of the global vaccines market with the availability of different formulations and technologies, according to the regulatory rules of each



country. It is consensual that the vaccination program can prevent reproductive diseases as BVDV, HoBiPev and BoHV-1 in dairy and beef herds, however it has a cost for dose, and this investment should be returned in protection. However, Brazilian scientific groups have reported some concerns about the effectiveness of BVDV vaccines because the great antigenic variability of BVD virus. The most commercial vaccines available in the Brazilian market contain virus strains isolated from other countries, and therefore these protocols may not be effective for viral strains circulating in Brazilian states (FLORES et al., 2018), in addition to the antigenic diversity among local isolates. The serological cross-reactivity between BVDV strains is low (FLORES et al., 2005). Most of the inactivated vaccines available in our country contain only BVDV-1, only a few formulations contain BVDV-2, and anyone commercial product includes HoBiPev.

HoBi-like species was first described by researchers in Europe in 2004, and it was isolated from a batch of fetal bovine serum imported from Brazil. Since then, there is a hypothesis that this virus is widespread in South America, especially in Brazil. However, the HoBi-like virus has also been detected from sera originating in Australia, Canada, Mexico and the United States (BAUERMANN, et al., 2013). Therefore, HoBi-PeV seems to represent another threat to animal production, and demands new vaccines formulations.

Fulton et al. (2020) evaluated the immune response of six commercial vaccines against BVDV, and 5 was composed by MLV. In this study, MLV vaccines containing the *Singer* strain induced higher levels of antibodies against BVDV-1 compared with vaccines containing the *NADL* strain. This study indicated that the strain reflects differences in the immune response against BVDV subgenotypes.



Baccili et al. (2019) conducted research evaluating three formulations with different adjuvants. Thirty five heifers were distributed into three experimental groups: Group I (G1) was vaccinated with a commercial vaccine containing inactivated strains of BVDV-1, BVDV-2 and BoHV-1 diluted in aluminum hydroxide as an adjuvant (n =9); Group II (G2) was immunized with a vaccine containing inactivated strains of BVDV-1, BVDV-2, BoHV-1 and BoHV-5 in oil emulsion as an adjuvant (n=10); Group III (G3) was vaccinated with a commercial vaccine containing BVDV-1 and inactivated BVDV-2 and modified live thermosensitive BoHV-1, diluted in adjuvant containing Oil A, Amphigem and cholesterol (n=10). In addition to the unvaccinated control group (n=6). G1 had the higher serological response for BVDV-1, however it was not observed seroconversion for BVDV type 2. Serological response against BVDV-2 was detected only in G3. Animals that received the oil emulsion (G2) showed a weak or undetectable response against both BVDV-1 and BVDV-2.

Recently study conducted by Silva, Weiblen & Flores (2021) investigated the neutralizing activity against HoBiPeV induced by two commercial vaccines, one attenuated and the other inactivated, and by three replicative experimental vaccines (VAC1 = monovalent, containing BVDV-1; VAC2 = bivalent, containing BVDV-1 and BVDV-2; VAC3 = trivalent, containing BVDV-1, BVDV-2 and HoBiPeV). Results indicated that formulations containing only BVDV-1 and BVDV-2, especially inactivated vaccines, may not provide protection against HoBiPeV. There are few studies presenting the serological response of reproductive vaccines and its cross-reaction with HoBiPeV.



Biosecurity has been defined by the World Health Organization and the Food and Agriculture Organization as “a strategic and integrated approach to analyzing and managing relevant risks to human, animal and plant life and health and associated risks for the environment” (RENAULT et al. 2021).

The objectives of biosecurity management program can be faced in three sides: animal, human and environment. The animal side includes the decrease risk of infection, morbidity, severity of diseases, treatments and mortality, in addition to the increase of animal welfare, expression of genetic potential, growth, development and performance. The implementation of biosecurity practices also reflects on some advantages for the humans like increase the animal protein availability, food safety and security, in addition to the decrease risk of zoonoses, antimicrobial resistance, and drug residues in milk and beef. In relation to the environmental concerns, biosecurity can turn in sustainability and decrease of environment contamination. Biosecurity also improves the global marketing, by the attendance of the international requirements imposed by large importing markets, and prevent restrictions related to sanitary barriers.

There are 5 levels of the biosecurity in animal production systems: (i) bio-exclusion, biosecurity measures (BSM) preventing the introduction of a pathogen at a farm, (ii) bio-compartmentalization, BSM preventing the spread of a pathogen within the farm, (iii) bio-containment, BSM preventing the spread of the pathogen to other farms or premises, (iv) bio-prevention, BSM preventing the spread of zoonotic pathogens to humans, and (v) bio-preservation, BSM preventing environmental contamination (RENAULT et al. 2021).



In our research and biosecurity projects we have been adopting the *Dairy Biological Risk Management* developed by CFSPH (2004). The CFSPH BRM program used the concepts of risk analysis: risk perception, risk assessment, risk management and communication. The general risk perception depends on five determinants: the risk perception in terms of probability and severity; the perception of the behavior benefits or outcomes; the perception of barriers to the behavior implementation; the outcome achievement; and the health motivation. Risk assessment is a tool to the detection of strengths and weaknesses related to a disease entering and spreading on the production system. Disease management practices should focus on minimizing identified risks. Communication is essential to the success of the biosecurity plan. People need to understand the project and support the implementation and changes routines on the farms (BICKETT-WEDDLE, 2019).

Cardwell et al. (2016) reported that the implementation of biosecurity practices reduces the risk of disease and infectious agents introduction through time. It was observed a tendency to decrease the prevalence of seropositive animals for BVDV in the 2<sup>nd</sup> and 3<sup>rd</sup> year after biosecurity program implementation.

Gates et al. (2013) conducted a study in 434 dairy and beef herds from Scotland. They applied a risk assessment to the detection of strengths risk factors for BVDV local dissemination. It was observed that the success of the BVDV control program can be hampered by the propagation of the agent through the commercialization of PI's animals, either by the sale of positive pregnant animals, or by positive fetuses in negative pregnant females, in addition by the low





compliance of producers in follow the recommendations provided regarding biosecurity.

Santman-Berends et al. (2015) established four different scenarios in Dutch dairy herds. The first involved testing and removing of PIs with consequent monitoring; the second scenario include only vaccination of the herd; the third scenario consisted of tracking and eliminating PI animals and monitoring newborn calves and finally, the fourth scenario identified the status of the disease in the herd and applied control measures for 3 years. After this period, the presence of PIs was investigated and positive animals were culled. In the scenario where tracing and removing of PI animals and monitoring of the subsequent status was obligatory, the benefit to cost (B/C) ratio was 1.5 (€1.5 benefit for each invested euro). In the scenario in which the BVDV status of all herds was determined, followed by voluntary measures before control measures became obligatory, the B/C ratio was 1.1.

To conclude, BVDV is a global concern, and its prevention and control depends on a tridimensional project involving PI screening, a good and effective vaccination protocol, and the implementation of biosecurity practices to maintain the herd healthy and free of BVD virus.



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## DIAGNOSTIC METHODS ACCURACY FOR BRONCHOPNEUMONIA DETECTION

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The diagnosis of bovine bronchopneumonia is a daily challenge. There are different types of diagnoses, the clinical diagnosis (to determine if a calf is sick or not) and the etiological diagnosis (the etiology of the infection). Practically, most of the emphasis is traditionally put on the clinical diagnosis. On a day-to-day basis, the clinical diagnosis of bronchopneumonia is generally performed by people without specific veterinary knowledge (eg producers or employees). In order to improve this process, various clinical scoring systems have been proposed with their advantages and limitations. The various clinical scoring systems that are available consist in systematically recording symptoms associated with bronchopneumonia (more commonly nasal and ocular discharge, cough, rectal temperature, ear position and breathing patterns). The calf total score is therefore compared with a specific threshold (treatment threshold) and a decision is taken. The theoretical advantages of these scores is to offer a stable and consistent definition of respiratory health event on a specific farm. This further helps to record farms' health events.

However, one of the main challenges to determine these tests' accuracy is the absence of an affordable and practical gold standard test to determine the true



respiratory status of the calf. The accuracy of any test generally consists in its ability to diagnose truly affected calves (sensitivity) and its ability to diagnose non-affected calves (specificity). Using different tests strategies would be helpful to rule in (positive result from a specific test) or rule out the disease (negative result of a sensitive test). Moreover, as for any test that is performed, it is important to determine the reliability of the scoring system that is used (variability of the score due to the rater vs true variability of the score in the calves). A specific teaching period should be always be dedicated to a proper teaching on how to assess the clinical signs included in the score. This would be particularly helpful especially because preliminary studies done on that topic have shown that inter-rater reliability may significantly vary. It is also important to remember that diagnostic test accuracy of these scores may potentially be impacted by specific pathogens present in the farm.

To date, the sensitivity and specificity of the two clinical scales that are commonly used (eg Wisconsin and California scoring systems) generally comes from North-American studies. The sensitivity varies between 50-70% sensitivity when performed in calves with no specific prior suspicion history whereas it rises to 70-95% when applied on calves with a specific suspicion. The specificity is generally between 70 and 80%. In specific Brazilian context, it has been shown that the sensitivity/specificity of the Wisconsin and California respiratory scores were 78%/82% and 67%/79% respectively. Other automatic detection tools such as behavior, drinking and eating characteristics or interaction of the calf with its pen mates are promising but, to date, their accuracy seems to be limited.



The clinical diagnosis of bronchopneumonia performed by veterinarians is generally performed after a thorough clinical examination. The stethoscope is another tool that is used daily by practitioners for the examination of the calves. However, data are scant and conflictual concerning its reliability and accuracy to diagnose bronchopneumonia. There is a wide variation between veterinarians examining the same animals. This may come from the fact that some respiratory sounds are considered as subjective (eg increased bronchial sounds). On the opposite, some auscultation signs may be more reliable (eg: crackles or wheezes) but at the cost of a lower sensitivity. Electronic stethoscope that wirelessly transmit information to a computer has recently been developed for feedlot calves. However, its usefulness for preweaned dairy calves auscultation is very limited.

Finally, thoracic ultrasound is maybe the diagnostic tool that has received the most attention in the last 10 years as a practical and affordable calf-side diagnostic test. The information provided by thoracic ultrasound is helpful to determine the extent of consolidated (ie non-ventilated) lung which is associated with various negative outcomes. Thoracic ultrasonography has the advantage of being reliable and rapidly performed with minimal equipment. Specific information on this ancillary test are given in another part of this symposium.

The etiological diagnosis of bronchopneumonia is also another challenge that would not be detailed in the current presentation. It is important to mention that most commonly encountered pathogens (eg bacteria) can be found in upper respiratory tract of healthy animals and that for PCR tests, false positive results



can be obtained when sampling is performed shortly after modified live vaccines administration.



## MICROBIOLOGICAL DIAGNOSIS OF BOVINE RESPIRATORY DISEASE (BRD)

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### Summary

Although bovine respiratory disease (BRD) can often be managed without diagnosis of microbial agents, when presumptive management is unsuccessful, identification of viruses or bacteria may reveal unexpected contributors, or agents for which a change in vaccination strategy or antimicrobial may be appropriate. The most common and feasible techniques for field use include nasal swabs, guarded nasopharyngeal swabs (NPS), transtracheal aspiration, and non-endoscopic bronchoalveolar lavage (BAL). Nasal swabs or NPS are ideal for rapidly sampling large numbers of cattle; evidence suggests that testing these for viruses or bacteria reliably represents the agents in the lower respiratory tract at the group level. If only one or a small number of animals are to be sampled, the tracheal aspirate or BAL may provide a more reliable result. Recent vaccination with modified live viral (MLV) vaccines can cause false positive results by any method, thus sampling should not be carried out within one month of MLV vaccination. Paired serology can be useful for diagnosis of viral agents, but serologic tests for bacterial BRD agents are not readily available in diagnostic





laboratories. Paired serology can be difficult to interpret in calves with maternal antibody, or in recently vaccinated cattle.

## Introduction

When dealing with an individual case of BRD, or a BRD outbreak, it is not always necessary to identify microbial agents. It is often reasonable to treat a case or an outbreak presumptively. If an operation has repeated BRD outbreaks, it is also reasonable to first assess management practices that may underly endemic or epidemic BRD, such as inadequate passive transfer in calves, crowded or dirty housing, poor ventilation, or inadequate nutrition. But if such factors are addressed, and disease persists, then assessment of the infectious agents involved may provide support for introduction of new vaccines, or may justify a change in routine antimicrobials used for treatment. Opportunities to gain information through routine necropsy of animals that die should not be missed.

### **First: confirm that respiratory disease is present**

It is important to understand that **the sampling methods reviewed in this paper are only appropriate for use in cattle confirmed to have BRD by clinical assessment, with or without additional tests to confirm the presence of lung disease.** The bacteria that most commonly contribute to the development of BRD--*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*, and *Trueperella pyogenes* (the last associated with chronic



abscessing pneumonia)—are all organisms that can also be commensals on the respiratory mucosa of healthy cattle. Thus, a diagnosis of BRD cannot be made simply by isolating any of these bacteria from the respiratory tract of one or more cattle. **Testing for bacterial BRD pathogens should not be undertaken without also testing to confirm the presence of lung disease** via clinical assessment, necropsy, ultrasound, radiography, and/or cytologic evaluation of tracheal aspirates or bronchoalveolar lavage (BAL). However, when isolated from respiratory materials collected from cattle with evidence of lung pathology, any or all of the agents can be presumed to contribute to disease. It is inappropriate to make a diagnosis of pneumonia in a calf with no evidence of respiratory disease simply because *Mannheimia haemolytica* or any of the other bacteria listed above was identified in materials collected from the respiratory tract. Moreover, confirmation of the presence of BRD by clinical signs alone may be misleading. Given that the sensitivity of clinical diagnosis has been estimated to be as low as 27%,<sup>15</sup> and the specificity has been estimated to be as low as 63%,<sup>19</sup> confirmation of the presence of lung pathology in one or more animals in an affected group, by necropsy, transthoracic ultrasound, or other methods, is advised before microbiologic testing is undertaken.

The interpretation of respiratory virus identification in cattle is more clear, because we do not currently understand any viruses to be “normal flora”. This may change in coming years as more study of the respiratory “virome” (all viruses that can be identified by sequencing DNA and RNA in respiratory tissue or secretions) is undertaken.<sup>12</sup> It may be that some viruses are essentially normal flora, but that has not yet been confirmed.



While cattle should not be sampled for microbiologic testing before the presence of BRD is confirmed, in groups where BRD has been confirmed in some cattle but not others, it can be useful to sample some cattle that have not yet been identified to have signs of BRD. Particularly in virus outbreaks, cattle in the pre-clinical stages of infection may be more likely to yield virus.

### **Effects of modified live organism vaccination on test results**

Before sampling cattle for microbiologic diagnosis, confirm that the cattle have not recently been vaccinated with modified live virus or bacterial vaccines. While vaccines delivered intranasally are most likely to be shed for at least several days post vaccination,<sup>18</sup> some live vaccines given by injection can also be identified in respiratory secretions collected from recently vaccinated cattle, particularly when PCR is used for diagnosis.<sup>18</sup> It is advised to wait at least one month after vaccination with modified live vaccines before attempting microbiologic diagnosis.



## **Antemortem sampling methods**

### *Nasal and nasopharyngeal swabbing*

Traditionally, nasal swabs have been used to sample cattle for respiratory viruses, but not bacteria, because identification of bacteria which can be commensals from the nasal passages has been of uncertain significance. Nasal swabs are collected with a cotton- or polyester (Dacron)-tipped swab approximately 6 inches (15 cm) in length. Cotton swabs may be superior for bacterial culture, while polyester swabs are superior for identification of many viruses. It is advised to wipe out the nostril to be sampled with a single-use disposable paper towel before swabbing, to remove dirt and excessive secretions that may be heavily contaminated. The swab should be inserted into the ventral meatus of the nostril for nearly the entire length of the swab, rubbed back and forth on the mucosa, then withdrawn. Obviously, restraint of the animal's head will be necessary. Do not touch the swab to the planum of the muzzle or other areas before or after sampling; transfer the swab immediately into the tube to be used for transport. If multiple animals are to be sampled, wear disposable examination gloves and change gloves between animals sampled to prevent transfer of agents from one animal to the swab collected from another animal.

Although nasal swabs have not traditionally been used for identification of bacterial BRD pathogens, in one study, the results of nasal swab culture for



identification of bacterial BRD pathogens were found to agree well with results of transtracheal aspiration and culture, in dairy calves with clinical signs of acute undifferentiated BRD.<sup>7</sup> This suggests that nasal swabs may be adequate to identify bacteria associated with BRD in clinically affected animals. Nasal swabs have been used in multiple studies to identify *Mycoplasma bovis* and other mycoplasmas.<sup>2,14,20</sup> However, some investigators have found overgrowth of contaminants to limit the value of unguarded swabs for identification of bacterial pathogens.<sup>16</sup> This observation led to the widespread adoption of guarded nasopharyngeal swabbing (NPS) as a method to obtain a sample thought to be more representative of agents causing BRD with less contamination.

Guarded NPS is completed with a long (26 – 33 inches, or 66 – 83 cm) single or double sheathed swab, of the type typically used for mare uterine culture. Versions that can be used include the Double Guarded Uterine Culture Swab (MAI Animal Health, Item #64300) or the Double Guarded Culture Swab (Jorgensen Labs Inc., Item #J0273). The available swabs are longer than needed for bovine nasopharyngeal swabbing, which makes them awkward for use. To collect a guarded nasopharyngeal swab, the head is restrained, and the nostril is wiped clean with a single-use disposable paper towel. The guarded swab with swab inside the guarding sheath is advanced into the ventral meatus to approximately 2 cm below the medial canthus of the eye. The inner sheath is then advanced approximately 2 cm, then the swab is advanced a further 1 cm, rolled 3 – 4 times, then withdrawn into the inner sheath. The inner sheath is then withdrawn into the outer sheath, and the entire guarded swab is removed from



the nostril. The swab is withdrawn from the distal aspect of the swab (that is, not pushed through the contaminated end), inserted into the transport tube, and the handle of the swab is cut with scissors or broken to a length that allows the tube to be capped. The swab should be moist but not bloody. Blood may be seen on the swab if the animal throws its head during sampling, or if the swab is advanced too far. If sampling multiple cattle and multiple swabs are bloody, the swabs have likely been inserted too far. The value of sampling with a guarded NPS is that the swab is protected from contamination with bacteria from the rostral nasal passages, thus fewer contaminants should be present on the plate if swabs are submitted for bacterial culture.

*In calves or cattle with clinical signs of BRD*, guarded NPS have been shown to agree moderately to very well with the results of samples collected from the lower respiratory tract by tracheal aspiration, tracheal swabbing, or bronchoalveolar lavage.<sup>1,6,7,11</sup> The agreement between guarded NPS and lower airway culture is generally better at the level of the group than at the level of the individual. Therefore, if only one or two calves or cattle with BRD are to be sampled, a transtracheal aspirate or BAL is likely to provide a sample that more reliably represents agents in the lower airways. However, when sampling multiple calves or cattle with signs of respiratory disease, the composite results of NPS culture of the entire group can be expected to represent the genus and species of bacteria that would be found with more invasive lower airway sampling. The agreement between NPS and lower airway samples for assessment of **antimicrobial susceptibility** of isolated bacteria has not been studied



extensively. In one study antimicrobial sensitivity testing of bacteria isolated from NPS cultures from a small number of cattle agreed moderately well with antimicrobial susceptibility testing of bacteria isolated from the lower respiratory tract.<sup>6</sup>

While guarded NPS can also be used to sample cattle for identification of viruses, it is not clear that this approach is superior to nasal swabbing. Because nasal swabbing is less cumbersome, and short nasal swabs cost less than the long, guarded swabs used for NPS, in most cases short swabs are likely to be more efficient for identification of viruses. However, in one study comparing the agreement between agents found on nasal swabs, NPS, or in BAL to the agents found in transtracheal aspirates in dairy calves with undifferentiated BRD, for BRSV the agreement between nasal swabs and tracheal aspirates was only moderate, and for respiratory coronavirus the agreement was even worse. When calves were positive for BRSV it was more often found in tracheal aspirates and BAL, while when calves were positive for coronavirus, it was more often found on nasal swabs and NPS.<sup>7</sup> While these results suggested that tracheal aspirates or BAL are more reliable than nasal swabs or NPS for the identification of BRSV in individual calves, nasal swabs have been used to identify BRSV in experimentally infected calves<sup>10</sup> and in natural outbreaks,<sup>8</sup> so when multiple calves are to be sampled in an outbreak, nasal swabs can be used to reliably identify BRSV in at least some of the animals.



### *Tonsillar washing*

Tonsillar washing has been used to identify *Mannheimia haemolytica*,<sup>9</sup> which appears to persist in the tonsillar crypts when the bacteria may not be present in high enough numbers to identify by routine nasal swabbing or NPS. The technique requires skill practice and is likely most useful for research applications.

### *Tracheal aspiration*

Secretions from the bronchioles and bronchi are ultimately expelled from the respiratory tract by movement up the trachea by the mucociliary apparatus. Thus, aspiration of fluid from the trachea is used to collect a composite sample of material from the lower respiratory tract, while avoiding contamination from the nasal passages and nasopharynx. The technique requires some practice for proficiency, it is invasive, and it takes the most time to complete. Thus tracheal aspiration may best be reserved for sampling individuals or small numbers of animals, when a sample certain to represent the lower airways is desired.

Tracheal secretions can be collected either by *transtracheal aspiration* or *endotracheal aspiration*. In the field, transtracheal aspiration is likely to be most





feasible. Kits are available for transtracheal aspiration (for example the Large Animal Trans-Tracheal Wash Kit, MILA International, Inc., Item #TW1228, or the JorVet Tracheal Wash Kit, Jorgensen Laboratories Inc., Item #J0283). An advantage of such kits is that they include a trochar to insert through the tracheal wall instead of a needle, which greatly decreases the chance that the catheter used to instill and recover fluid will accidentally be cut off inside the trachea. Alternatively, materials can be purchased individually and used to create home-made kits; a 12- or 14-gauge 2 inch needle and a polypropylene tube of appropriate diameter and length to insert through the needle can be used satisfactorily. For very small ruminants (such as small calves, lambs, or kids) a through-the-needle intravenous catheter with a small gauge needle can be feasible for transtracheal aspiration. The catheter to be threaded through the needle that is passed into the trachea should be long enough to reach the thoracic trachea, where the trachea is horizontal and instilled fluid can be aspirated more easily.

To perform a transtracheal aspirate:

- 1) Restrain the animal with the head in extension, nose pointing upward. For large cattle it can be helpful to place two halters, one tied on each side, to center the head;
- 2) Clip and aseptically prepare a site approximately 12 cm long by 8 cm wide, directly over the ventral aspect of the trachea, about one-third of the distance between the larynx and thoracic inlet.
- 3) Instill 3 to 5 ml of 2% lidocaine subcutaneously at the entry site.



4) Wearing sterile gloves or exam gloves, make a full-thickness stab incision with a scalpel blade at the site where the trochar/needle will be inserted.

5) While using the non-dominant hand to stabilize the trachea (to prevent it from moving side-to-side), use the dominant hand to firmly push the trochar/needle through the tracheal wall. This may take some force in large cattle. Once the needle pops through the tracheal wall, air may be heard moving through the trochar/needle.

6) Push the hub of the trochar/needle up so the end of the trochar/needle that is inside the trachea is directed downward, then thread the catheter through the needle to the thoracic trachea. Depending on the size of the animal, instill 20 – 60 ml of sterile isotonic saline as quickly as possible, then aspirate back repeatedly, moving the catheter in and out while aspirating. Often no more than 10% of the fluid instilled is recovered. The rest of the fluid will be coughed out or absorbed.

7) Transfer the fluid to a sterile tube. Divide the sample into 2 aliquots, one for culture and virus identification, and one for cytologic evaluation.

8) Remove the cannula/needle and catheter. Ideally a pressure wrap is placed over the site for 24 hours to limit subcutaneous emphysema, but this is not absolutely necessary.

Instillation of fluid may induce some coughing, which can help to increase the volume of fluid recovered. However, forceful repeated coughing can cause the cannula to be flipped up into the pharynx. If this occurs, fluid may come out of the mouth when instilled, and the sample will not be useful for diagnosis, as it will be



contaminated with oral bacteria. If this occurs, remove the cannula/needle and catheter and repeat the process. Administration of an intravenous dose of butorphanol may decrease paroxysmal coughing.

The fluid recovered from a transtracheal aspirate is transferred aseptically to a sterile tube and submitted for identification of viruses and/or bacteria as described below. Ideally a subsample of the fluid is placed into a second tube for submission for cytologic evaluation. **Because the trachea is not a sterile site, simply culturing bacteria from a tracheal aspirate does not indicate bacterial respiratory infection.** The presence of cytological signs of confirms the presence of an inflammatory response and supports a diagnosis of infection. Alternatively, if clinical signs or ultrasound evaluation indicate lung disease, the cytologic evaluation can be omitted. **If fluid collected by transtracheal aspiration is to be submitted for cytologic evaluation, contact the laboratory to determine how the sample should be prepared before shipment.** Cells deteriorate quickly in saline, and so it may be advisable to make some direct smears onto glass slides to send with the tube of fluid for cytologic evaluation. Alternatively, slides can be made and stained with Diff-Quik and read in house.

Although tracheal aspiration is invasive, complications are uncommon. The trachea readily clears small amounts of fluid and bacteria, so it is unlikely that respiratory infection will be induced. More common complications include: 1) abscessation at the site where the needle was introduced (particularly if the



trochar/needle is removed before the catheter, so that bacteria in the trachea are pulled through the subcutaneous space as the catheter is removed). 2) Subcutaneous emphysema on the neck and thorax; this is typically seen for 24 – 48 hours after the procedure and should resolve without intervention. 3) Cutting or breaking the catheter during the procedure, so that the distal aspect of the catheter is left in the trachea. This is alarming, but the animal should eventually cough the catheter out in the hours after the procedure. If the animal is placed in a stall or pen without bedding, the end of the catheter can often be found lying on the ground several hours later, after it is coughed out (although the animal may also chew and swallow the catheter after it reaches the pharynx). The catheter fragment can also be removed with an endoscope if one is available. Hypoxia and exacerbation of respiratory distress can be induced in patients with severe lung disease and respiratory compromise, and the procedure should be avoided in such patients.

If the veterinarian has access to an endoscope, endotracheal aspiration can also be used to collect a sample of material from the trachea.

### *Bronchoalveolar lavage (BAL)*

Bronchoalveolar lavage (BAL) yields a sample of fluid from a single bronchoalveolar unit. Thus, BAL provides a sample from the deepest region of the lung, but from only one relatively small area of the lung. Therefore BAL is most appropriate when lung disease is diffuse, as the sample collected may not



represent the diseased area of lung if the process is localized. Consistent with this, a recent report found poor correlation between the presence of lung consolidation identified by transthoracic ultrasound evaluation and cytologic evidence of inflammation in BAL fluid in calves.<sup>17</sup> In spite of this, BAL has been used by some investigators to identify evidence of inflammation, and/or microbial agents associated with BRD, in calves with bronchopneumonia,<sup>13</sup> which is not necessarily diffuse.

A BAL can be collected by a tube inserted blindly through the nose and advanced into the lung. BAL can also be collected through an endoscope of sufficient length to wedge into a bronchus, but in most cases this will require a 2- meter to 3-meter endoscope, which most veterinarians in practice are unlikely to have. A BAL collected by a tube inserted blindly through the nasal passage is feasible for collection in the field. Tubes for BAL can be purchased (for example, Large Animal Broncho-Alveolar Lavage Catheter, MILA International, Inc., Item #BAL240, or Broncho-Alveolar Lavage Catheter, Jorgensen Laboratories, Inc., Item #J0639). Because it is not usually feasible to sterilize tubes quickly in the field, if multiple animals are to be sampled, a separate tube will be required for each animal. The tubes can be re-used, but it is necessary to clean and sterilize the tubes between uses.

The basic approach to perform a BAL is:

- 1) Place a halter on the animal and restrain the head in extension, with nose pointed upward.



- 2) Wipe the nostril clean with a disposable paper towel. While not necessary, it can be helpful to instill 2 – 3 ml of 2% lidocaine into the nostril, to decrease irritation of tube passage.
- 3) Advance the tube into the nasopharynx and through the larynx and into the trachea. This can be challenging; if the tube is swallowed it will enter the esophagus and be contaminated. It can be helpful to advance the tube quickly at the time of inspiration, when the larynx is open most widely. Occasionally it can be helpful to instill 3 - 5 ml of 2% lidocaine through the tube into the nasopharynx at the level of the larynx to numb the larynx. Once the tube is advanced into the trachea, coughing may occur. The placement of the tube in the trachea can be confirmed by shaking the trachea side-to-side, which can cause the tube to palpably rattle in the trachea. Once in the trachea the tube should be advanced rapidly until it is wedged into place, and can be advanced no further. If the tube is cuffed, the cuff should then be inflated.
- 4) Depending on the size of the animal, 60 to 180 ml of sterile isotonic saline is instilled through the tube, and then the fluid is withdrawn. A good sample will be cloudy with grossly visible foam due to alveolar surfactant. Hemorrhage is not expected. Approximately half the volume instilled is typically recovered; the remainder of the fluid will be coughed out or absorbed.
- 5) If inflated, the cuff should be de-flated, then the tube is removed.



- 6) The sample should be transferred to tubes as described for tracheal aspirate. As described for tracheal aspiration, if cytologic evaluation is to be completed, the fluid should be processed the same day.

Complications of BAL are uncommon; these include introduction of infection, trauma to the pharynx, hemorrhage, and induction of bronchospasm and/or hypoxia in patients with severe lung disease and respiratory compromise. These can be avoided by using clean and careful technique. The procedure should be avoided in animals with significant respiratory distress.

### *Thoracocentesis*

Infection with *Mannheimia haemolytica* or *Histophilus somni* or, less commonly, other agents, may cause pleuropneumonia, leading to pleural inflammation and effusion. Other disease processes, such as neoplasia, can also cause pleural effusion. A sample collected from inside the airways is in most cases adequate for microbiologic diagnosis of pleuropneumonia. However, in cases with pleural effusion it may be desired to collect a sample of pleural fluid for microbiologic and/or cytologic evaluation via thoracocentesis.

The basic approach to thoracocentesis is:

- 1) Clip and aseptically prep a site over the 5<sup>th</sup> – 7<sup>th</sup> intercostal space, at the lowest point in the fluid (typically approximately 4 – 6 cm above the point of the olecranon). On the left side, take care to avoid the heart.



If an ultrasound machine is available the location of the fluid can be easily confirmed. Percussion may also be used to identify the location of pleural effusion.

- 2) Instill 2 to 4 ml of 2% lidocaine subcutaneously at the site to be sampled. Remember that the needle or teat cannula will be inserted immediately in front of the rib, to avoid hitting blood vessels and nerves behind the rib.
- 3) Apply sterile gloves and use a scalpel blade to make a full-thickness stab incision through the skin. Use sterile gauze to wipe away blood.
- 4) Insert the male end of a sterile extension set into the female end of a sterile teat cannula, then attach a 10 to 30 ml syringe into the female end of the extension set. This will prevent air from entering the pleural space when the teat cannula is inserted into the pleural space. A 5 cm 14-gauge needle could be used instead of the teat cannula to enter the pleural space, but **a needle is more likely to lacerate the lung, possibly inducing serious pneumothorax.**
- 5) Using firm pressure, push the teat cannula through the thoracic wall, entering the thorax right off the front of the rib. Once the teat cannula has been advanced to the hub, aspirate with the syringe to withdraw fluid. Process fluid as described for tracheal aspirate.

Complications of thoracocentesis are uncommon but include abscess or hematoma formation at the site of centesis, or pneumothorax due to entrance of air through the teat cannula, or due to laceration of the lung. Abscess or





hematoma at the site of centesis can be managed symptomatically. If care is taken to minimize entrance of air into the pleural space the degree of pneumothorax induced is negligible. The most serious complication is lung laceration, which can be avoided with proper restraint and by using a blunt-ended teat cannula instead of a needle for aspiration.

#### *Transthoracic lung biopsy*

Transthoracic lung biopsy may be considered the antemortem sampling approach of last resort for the diagnosis of lung disease. This technique is invasive, and complications that can be life-threatening or fatal are not rare. The procedure is most appropriate when diffuse lung disease is present, or when ultrasound can be used to confirm the exact site of abnormal lung to be sampled, because the sample collected is quite small relative to the size of the lung. Approaches have been described.<sup>3,4</sup> A biopsy is collected using a Tru-Cut or similar biopsy instrument through a stab incision at a site over an intercostal space that has been clipped, aseptically prepped, and locally anesthetized. Biopsies are usually collected from the dorsocaudal lung, where blood vessels and airways are smaller, and damage to the structures is less likely to lead to severe complications. Complications include pneumothorax or pulmonary/pleural hemorrhage that can be severe. In one study,<sup>5</sup> transthoracic lung biopsy was evaluated for identification of early lung disease in feedlot cattle, but the technique did not identify information in enough cattle to be considered



useful. In referral institutions, lung biopsy can also be performed via thoracoscopy, which may be safer though relatively very expensive.

### *Serology*

Serologic testing can be used to confirm infection with agents associated with BRD. Most diagnostic laboratories offer serologic testing to identify serum antibodies to viruses but not bacteria. Antibody titers to the bacteria that commonly cause BRD can be difficult to interpret and are generally reserved for research applications.

Identification of infection with respiratory viruses commonly associated with BRD is best accomplished using paired serology, as a one-time test can be difficult to interpret, because vaccination or past exposure may lead to the presence of antibodies that do not necessarily indicate recent exposure. Identification of a 4-fold increase (that is, 2 2-fold dilutions) in antibody titer between an acute and a convalescent sample (collected 2 – 4 weeks after the acute sample) suggests recent infection. Note that a 4-fold *fall* (or decrease) in titer also suggests recent infection. Because the significant change in titer may be missed in a single individual, paired serology is most useful for identifying group-level infections, by sampling multiple animals. The acute samples should be collected at the time of the respiratory outbreak, and the serum should be stored in the freezer until the convalescent sample is collected, and both samples are sent for testing at the same time. This is important because day-to-day variations in serologic tests



could lead to misleading differences between the acute and convalescent samples if they are run on different days.

The results of serologic testing of calves that are likely to have maternal antibodies are particularly difficult to interpret. This is because maternal antibodies may prevent calves from seroconverting, even when they are infected. Thus failure to seroconvert in calves with maternal antibodies does not rule out infection. Moreover, it is not possible to differentiate maternal antibodies from the calf's own endogenously generated antibodies. Recent vaccination in cattle of any age can also make interpretation of serologic testing difficult.

### **Sample handling before and during transport to the diagnostic laboratory**

Whether collecting samples for identification of viral or bacterial pathogens, before traveling to the operation for sample collection, it is best to contact the laboratory where the samples will be tested in advance to determine the preferred transport medium to be used, and to determine the best temperature for sample storage. The information may be available on the laboratory's website. Identification of *Histophilus somni*, *Mycoplasma bovis*, or other mycoplasmas are particularly likely to require special medium for transport. The laboratory staff can provide good advice re the best sampling handling methods for the agents to be identified. Note that the laboratory will most likely not attempt to identify *Mycoplasma bovis* or other mycoplasmas unless this is specifically requested. In



many cases the laboratory can provide transport media, or can provide information about where transport medium can be purchased.

In general, swabs collected for isolation of viruses should be collected into transport medium that is specifically formulated for virus identification. Such media will usually contain antibiotics to inhibit growth of bacteria which could interfere with viral identification. Transport tubes for bacterial culture should not be used to transport swabs for virus identification, as overgrowth of unwanted bacterial contaminants is more likely in medium designed for bacterial transport.

When either viruses or bacteria are to be identified by PCR, inserting swabs into dry tubes is usually acceptable. Swabs in dry tubes can then be stored on ice packs, or in the refrigerator or freezer, before sending to the laboratory for PCR. The exact storage temperature to be used is more critical if virus isolation or bacterial culture is to be attempted, because some viruses and bacteria do not tolerate freezing, while others do not tolerate refrigeration, or storage at room temperature. Communicate with the diagnostic laboratory to determine the best storage and shipping temperatures before collecting samples for virus isolation or bacterial culture.



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## VACCINATION OF CALVES WITH MATERNAL ANTIBODY: IS IT EFFECTIVE?

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### Summary

Ensuring health of preweaning calves requires attention to colostrum intake and nutrition of the dam and calf, and targeted use of vaccination. Colostrum intake that leads to optimal, and not just adequate, passive immunity can improve calf health. The neonatal calf has a functional immune response, and calves can respond to vaccination in the first week of life, although maternal antibodies may suppress the response to early life vaccination. However, maternal antibodies do not always suppress response to vaccination, and many research studies have demonstrated benefit for calves vaccinated in the face of maternal antibody when they are between 30 and 120 days of age. The intranasal route of vaccination may prime immunity more effectively than the parenteral route if calves are vaccinated in the first week or two of life, when maternal antibodies are at the highest concentration. In calves, booster doses are important to ensure immunity, as not all calves will respond optimally at the time of their first vaccination. Field trials indicate that preweaning vaccination can sometimes improve health postweaning, but few field trials have evaluated the effects of preweaning vaccination on preweaning disease. Challenge studies and research





measuring immune function provide support for vaccination to prevent preweaning disease, but the evidence quality is not strong. More large field trials testing vaccination of preweaning calves to prevent preweaning disease are needed.

### **Immune Responses in the Newborn Calf**

An effective immune response is the foundation for health and growth in calves. For the calf, good dam health and nutrition, attention to colostrum intake, nutrition and timely use of vaccination all support immunity.

The concept that calves are immunosuppressed at birth, or that they lack immune competence, is often discussed in the context of managing calves for health and growth. However, it is important to remember that this is relative, but not absolute. While immune responses in a calf that is immunologically naïve are generally slower or lower than the same responses in a naïve adult cow, multiple research studies have demonstrated that calves can mount an adaptive immune response to vaccination in the first week of life, and even on the first day of life. In fact, the fetal calf is immunocompetent *in utero*; this is demonstrated by the fact that a calf infected *in utero* may be born with antibodies to the infectious agent which can be measured in serum collected before the calf consumes colostrum. Vaccination of calves against *Mycobacterium bovis* on the first day of life induced protection against disease due to challenge 15 weeks later.<sup>1</sup> Colostrum-deprived Holstein calves exposed to live coronavirus orally and intranasally on the first day of life



were protected from disease following challenge 21 days later.<sup>10</sup> When calves were vaccinated with ovalbumin (a model antigen for assessment of humoral immunity) at 2 days of age, antibody to ovalbumin was identified in serum of vaccinated calves 4 weeks later. Moreover, vaccination of calves at 2 days of age with bacille Calmette Guerin (BCG) led to skin test responses at 7 weeks of life.<sup>16</sup> This work demonstrated that both humoral and cell-mediated adaptive immune responses are functional in 2-day-old calves. However, calves with specific antibodies present in serum at the time of vaccination had depressed responses, indicating that serum passively derived antibodies could suppress the response to vaccination at 2 days of age. Taken together, these studies indicate that the calf's immune response is functional and can respond to vaccination as early as the first 2 days of life, but that the response can be depressed by the presence of specific antibodies.

### **Transfer of Passive Immunity via Colostrum**

While adaptive immune responses to vaccination as early as the first day of life have been demonstrated in calves, there is no question that calves lacking passively acquired maternal antibodies are more likely to become sick, and more likely to die, as compared to herdmates that obtain adequate passively acquired antibodies. This repeatedly demonstrated fact confirms that the functional immune response of the newborn calf is inadequate to provide optimal protection against infection in the first weeks of life. When failure of transfer of passive immunity (FTPI) is defined by a serum IgG concentration less than 800 or 1,000



mg/dl, studies have found that between 6% and 23% of beef calves have FTPI.<sup>4,18,23</sup> However, current recommendations are that optimal transfer of passive immunity is defined by a serum IgG concentration of 2,400 mg/dl or greater, and one large study found that 46% of 1,556 beef calves had suboptimal transfer of passive immunity. In one example, beef calves with serum IgG concentrations of less than 2,400 mg/dl were 1.6 times as likely to become sick, and 2.7 times as likely to die, as calves with serum IgG concentrations of 2,400 mg/dl or greater.<sup>4</sup>

Recent research has clarified the factors that impact transfer of passive immunity in beef calves. In a crossbred cow-calf herd, calves that failed to nurse within 4 hours of birth had a 2.8 times greater odds of morbidity before weaning ( $P = 0.03$ ), compared to calves that nursed within 4 hours of birth.<sup>11</sup> In this study, of the 77 cow-calf pairs studied, 3 calves died prior to weaning, and all failed to nurse by 4 hours of life. Of a variety of factors measured at 10 minutes after birth, a strong suckle reflex best predicted that a calf would nurse within 4 hours, and the combination of calving ease score (scored as unassisted, easy or difficult) and nursing within 4 hours best predicted whether a calf would have optimal transfer of passive immunity (defined by serum IgG of greater than 2,400 mg/dl). Importantly, there was no difference in the antibody concentrations of colostrum of cows with calves that failed to acquire optimal passive transfer and cows with calves that did, indicating that failure of transfer of optimal immunity was not due to inadequate quality colostrum, but was rather due to failure of the calf to ingest enough colostrum in time to absorb colostral antibodies.<sup>11</sup> In related work, this



research group also showed that beef calves fed 1.4 L of colostrum with moderate concentrations of antibody (70 g/L) by esophageal feeder within 1 hour of birth went on to nurse from their dams at a statistically significantly earlier time, compared to calves that were fed 1 L of colostrum with 100 g/L IgG, or calves fed 2 L of colostrum with 100 g/L IgG. Regardless of treatment, all but 1 of the 39 calves included in the study achieved optimal transfer of passive immunity (serum IgG 2,400 mg/L or greater), with the remaining calf achieving adequate transfer of passive immunity (serum IgG greater than 1,000 mg/dl).<sup>9</sup> The interpretation of these investigators was that if calves are fed colostrum with an esophageal feeder, as long as the colostrum contains at least 70 g/L of IgG, the calf should achieve at least adequate transfer of passive immunity, and may stand and nurse on its own faster than calves fed a larger volume of colostrum, which could improve cow-calf bonding.

### **Vaccination of Preweaning Calves: Effects of Maternal Antibody**

Historically veterinarians were taught that young calves cannot be effectively vaccinated because maternal antibodies would block vaccine responsiveness. However, many studies have shown that calves can, in fact, have a useful immune response to vaccines given in the face of maternal antibodies (IFOMA). Vaccination of calves IFOMA has been shown to induce an anamnestic (memory) response when they are boosted later in life,<sup>15</sup> vaccination IFOMA can prolong the persistence of antibodies,<sup>8</sup> and vaccination IFOMA can prime for T cell



responses even when calves do not seroconvert.<sup>5</sup> Intranasal vaccination may be superior to parenteral vaccination in calves with high concentrations of circulating antibodies;<sup>12</sup> however, the duration of immunity provided by intranasal vaccination of calves with circulating maternal antibodies may not last for more than a few weeks. Calves vaccinated intranasally with a modified-live BRSV vaccine had decreased lung pathology after challenge at 9 weeks post vaccination, compared to controls; however, when calves were challenged at 14 weeks after vaccination, there was no difference between groups.<sup>6</sup> In general, reports describing positive outcomes to vaccination of calves IFOMA have most often used 2-dose (priming and later booster dose) regimens with modified-live virus vaccines. While more variable, some inactivated vaccines have been effective; the effect of both modified-live and also inactivated vaccines in calves with maternal antibody is influenced by the type of adjuvant contained in the vaccine, as has been recently reviewed.<sup>3</sup> Vaccination of calves in the first month of life, when maternal antibodies are highest and the calf's immune system is the most immature, is least likely to be reliable. For example, a large clinical trial showed that vaccination of dairy calves with maternal antibodies at 2 and/or 5 weeks of age did not decrease respiratory disease in the first 90 days of life. However, it should be noted that the majority of calves treated for respiratory disease in that study were treated before 5 weeks of age, before the vaccination regimen had been completed for all calves.<sup>24</sup> A thorough review on vaccination of calves IFOMA has recently been published.<sup>26</sup>



## Effective Use of Vaccination in Calves With Maternal Antibody

All licensed vaccines have been shown to decrease disease post challenge, by some measure, in calves vaccinated prior to experimental challenge, as compared to unvaccinated control calves, as this is a requirement for licensure in the United States and other countries where the same or similar vaccines are used. However, most if not all licensing trials are completed in seronegative calves, which does not represent very well the way vaccines need to be used in the field. In practice, waiting to vaccinate calves until they are all seronegative is not necessary, as described above; moreover, it exposes calves to the possibility of disease when they become seronegative. Ideally the first priming doses of vaccine is administered while calves still have some level of passive immunity, which may at least be partially protective against disease, but not enough to completely suppress the response to vaccination. Such an approach has the potential to keep calves from ever becoming completely seronegative, improving their resistance to disease.

The fact that a population of preweaning calves will have a range of antibody titers at the time they are first vaccinated means that some calves will receive their first dose of vaccine when they are able to respond, while others will not be able to respond, due to very high concentrations of maternal antibody, or perhaps other factors. For example, a group of over 2000 Angus calves were vaccinated twice with a modified-live vaccine containing bovine herpesvirus-1 (BHV-1),



bovine viral diarrhoea virus 1 and 2 (BVDV1 and BVDV2), parainfluenza type 3 virus (PI3V) and bovine respiratory syncytial virus (BRSV). Calves were randomly assigned to be vaccinated either 3 weeks before weaning, and at weaning, or at weaning, and 3 weeks later. As measured by change in serum antibody titer, greater overall antibody response to BVDV1 and 2 was seen when calves received the first dose of vaccine at weaning, while greater overall antibody response to BHV-1 and BRSV was seen when calves received their first dose of vaccine 3 weeks before weaning.<sup>14</sup> The reason for this difference could not be determined by the study as designed. Importantly, although antibody titers were measured in all calves at four time points, of the over 2,000 calves evaluated, 107 calves had a BVDV1 titer of 0 at all timepoints tested, and 227 calves had a BHV-1 titer of 0 at all timepoints tested. This demonstrates that in a population of calves, there may be a small proportion of calves that never respond to vaccination. The variability in responsiveness of calves in a population to vaccination at any one time point is another justification for administration of booster vaccines, which give calves two opportunities to respond to vaccination.

The highest quality of evidence to support a clinical practice comes from meta-analyses and systematic reviews of multiple well-designed randomized controlled field trials. Unfortunately, few field trials have been completed that test the effect of vaccination of preweaning calves to prevent or decrease naturally-occurring disease. Preweaning, calf diarrhoea and respiratory disease are the two syndromes most often addressed by vaccination; at the time of this writing there are apparently no published systematic reviews specifically focused on



evaluating clinical trials testing the efficacy of vaccination to prevent diarrhea or respiratory disease in preweaning calves. Also, in trials that have been published, lack of a negative control group in some trials means that it is not possible to determine whether vaccination is better than no vaccination. A small field trial that included a negative control group found numerically, but not statistically, significantly decreased preweaning respiratory disease treatment risk in beef calves vaccinated at 3 and 5 weeks of age against *Histophilus somni*, *Mannheimia haemolytica*, and BRSV, compared to calves receiving no vaccination.<sup>22</sup> The small numbers of calves in each group (n = 26 - 29) may have decreased the power of this trial to identify significant differences. A clinical trial testing vaccination to prevent respiratory disease in preweaning beef calves showed evidence of benefit to decrease morbidity in the postweaning feedlot phase,<sup>13</sup> while another trial by the same group did not show an effect of preweaning vaccination to decrease postweaning respiratory disease.<sup>20</sup> A small trial assessing the effect of vaccinating cows in late gestation on respiratory disease in their calves prior to weaning showed a tendency toward decreased disease in heifer calves born to vaccinated dams.<sup>19</sup>

While no systematic reviews have evaluated the effect of vaccination on disease in calves with maternal antibody, two recent systematic reviews assessing vaccination to prevent bovine respiratory disease in feedlot cattle.<sup>17,21</sup> One of these reviews<sup>21</sup> concluded that the published trials were so variable that, while there was support for some viral vaccines to decrease bovine respiratory disease, the support was not strong; the second systematic review concluded that the





variability in published clinical trials made it impossible to identify a positive effect of vaccination to prevent respiratory disease in feedlot cattle.<sup>17</sup> If veterinarians are to learn more about the true effects of vaccination to decrease disease in calves with maternal antibody, more well-designed controlled field trials containing a nonvaccinated control group need to be completed. A helpful narrative review of evidence for various vaccination strategies used in cattle populations was recently published.<sup>2</sup> In the absence of systematic reviews, challenge studies and assessments of immune response indicate that vaccination of calves with maternal antibody may improve resistance to disease. Ranchers with perennial problems with preweaning beef calf pneumonia at 3 to 5 months have anecdotally reported decreased disease following institution of a program of intranasal modified-live viral vaccination in the first month or two of life, followed by a parenteral modified-live viral booster one to two months later, with the booster being given approximately one month before respiratory disease onset is expected. A recent challenge study indicated that an inactivated booster following intranasal priming could improve protection against disease in calves challenged with BRSV.<sup>7</sup>

## Conclusions

Opportunities exist for improving immunity in calves by ensuring optimal, and not just adequate, passive immunity through timely colostrum intake. At this time, data from challenge studies and research evaluating immune responses post vaccination provides some support for vaccination of calves with maternal



antibody to prevent disease. More field trials are needed to confirm the effect of vaccination in the face of maternal antibody on health and production in calves as the vaccines are actually used on farms and ranches.

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## VACCINATION PROTOCOLS AGAINST BOVINE RESPIRATORY COMPLEX- LIVE VS INACTIVATED, NASAL VU INJECTABLE AND TIMING

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### **Introduction**

Vaccination is an important component for the prevention and control of disease in cattle. However, too often vaccines are viewed as a catch all solution for management and nutrition errors; the “best” vaccine can never overcome these deficiencies. Proper vaccination in the young and developing heifer is the key to long term development of that animal as a reproductive unit in the herd. Modified live vaccines (MLV) have been used because of the good antibody response, longer duration of immunity, fewer doses needed per animal and lower cost. However, non-adjuvanted MLV vaccines fail to booster well vaccinated animals as active vaccine induced immunity neutralizes vaccine virus preventing the MLV from replicating and preventing a booster immune response. Improved adjuvants have increased the scope and duration of both MLV and inactivated virus immunity. The periparturient period (the last 3 weeks prior to calving and the first 3 weeks following calving) are poor times to initiate an immune response—hormonal, dietary and metabolic factors limit immune responsiveness. Postpartum is also a difficult time to vaccinate as lactation energy demands



supersedes immunity. Each vaccine program needs to be based designed based on animal flow, actual “disease” threats and labor on the farm.

### **What? Types of vaccines and pathogens/immunogens**

#### MLV and Inactivated- Together is even better

Modified live virus (MLV) vaccines have been used because of the good antibody response, longer duration of immunity, fewer doses needed per animal and lower cost. To lesser extent modified live bacterial vaccines have also been used (*Brucella abortus*, *Mannheimia hemolytica* *Pasteurella multocida*, *Salmonella dublin*). These ML vaccines are administered intramuscular, intranasally or subcutaneously. As the basis for establishing a good immune response, they are the best. Although the return to virulence in MLV vaccines has been minimal, mutations will occur and there is some risk of new strains arising. Non-adjuvanted MLV vaccines also fail to booster well-vaccinated animals. Active vaccine immunity neutralizes vaccine virus preventing the MLV from replicating and preventing a booster immune response. Unlike maternal interference, this active immune interference **never goes away** in well vaccinated animals. The animal’s immune system can’t differentiate between a natural infection or vaccine virus. Another issue with MLV IBR (BHV-1) vaccines is that they result in latency and their continued use throughout the life of the animal will insure that BHV-1 will be present in the herd even though the rates of shed are between the 0.13 and 2.6% of the animals shed.





Inactivated vaccines contain chemically or physically treated bacteria, toxins and/or viruses. There is no danger of replication in the vaccinated animal of the pathogen or adventitious agents that maybe present in a MLV. Improved adjuvants have increased the scope and duration of inactivated virus immunity. They have several disadvantages including cost, and more doses required per animal. Inactivated vaccines generate cell-mediated responses. Interestingly there is ample evidence that inactivated vaccines can effectively boost MLV vaccines. Inactivated vaccines have also been shown to decrease BHV-1 latency shed rates.

### **What to vaccinate for? What pathogens make sense?**

Cattle vaccine programs are probably the most effect against viral pathogens [bovine herpesvirus 1 (BHV-1; IBR) bovine respiratory syncytial virus (BRSV) and bovine viral diarrhea virus (BVDV)] This is because many of the cattle bacterial pathogens (*Histophilus somni*, *Mannheimia. hemolytica*, *Pasteurella. multcida*, *Moraxella spp.*, *Mycoplasma bovis*, *Salmonella typhimurium*, *Clostridium perfringens*) are “normal inhabitants” of the bovine microbiome and they are “endemic” in most herds. Stressors that are discussed below play a major role in allowing these “normal” bugs to become pathogenic. Looking at a herd it essential to have a strong diagnostic program in place to get accurate pathogen diagnosis. With next generation sequencing, diagnostic PCR and good old fashioned pathology and microbiology isolation there has never been a better time to determine which pathogens are occurring and when. Being strategic in



vaccination requires targeting those pathogens on that farm or ranch. Another term that we have learned from COVID19 is Replication Rate called  $R_0$ . Replication rate is the number of susceptible animals that one infected animal can infect. Probably one of the most “infectious” viruses is BRSV. BRSV has been estimated to have a  $R_0 \sim 36$ . A BRSV susceptible animal (neonate) are highly susceptible to BRSV infection because of the high  $R_0$ . In a herd with BRSV disease history, BRSV vaccination would be on the top of the list. Once an animal is infected with BRSV and endemic, the immunity is not perfect but  $R_0$  is 1.1 so it BRSV is barely circulating in the herd. For IBR and BVDV transient infections, the rate is around  $\sim 3$  meaning one infected animal shedding virus could potentially infect 3 susceptible animals. By the time we get 70-80% of the animals either infected or protected from vaccination the occurrence of infections to those viruses will be low and herd immunity has been achieved. The BVDV PI animal is the one case that totally destroys the concept of herd immunity. Since the BVDV PI animal continually sheds virus any susceptible animal is at risk of infection, This makes the  $R_0$  for a herd with BVDV PI of  $\infty$  “infinity” indicating that a herd with a PI animal can never vaccinate their way out of the threat of BVDV. Endemic viral infections frequently include rotavirus and bovine coronavirus along with *C. perfringens* represent a threat to the newborn susceptible animals. Environmental pathogens like *Bacillus anthracis* (anthrax), *Leptospira* spp, *E. coli*, *Campylobacter* require considerations based on herd history and locality. Finally, *Brucella abortus* represents a “regulatory” vaccine.



## **When do we vaccinate- age and stressors**

### **Age**

#### *Neonatal Calves*

The newborn calf is immunological naïve at birth. It has had no chance to enhance adaptive immunity by “experience” because of the protective environment in the uterus. It is further handicapped by maternal factors and the hormonal influences of parturition, and by its lack of antibodies in circulation and in the tissues. The ingestion of colostrum is essential for providing the neonate with immunological protection during at least the first 2-4 weeks of life. While all the essential immune components are present in the neonate at birth, many of the components are not functional until the calf is at least 3 weeks of age and may continue to develop until puberty.<sup>6</sup> This ongoing maturity of the immune system in the developing neonate coupled with maternal antibody interference makes vaccination strategy more complex. The mucosa epithelium provides immune function very early, making intranasal and oral vaccines effective in calves less than a week of age. Parenterally administered MLV vaccine responses begin at 7-10 days following birth although BVDV MLV vaccines should be avoided particularly in dairy calves before at least two months of age as the major BVDV vaccine strains inhibit innate immune bacterial killing for 10-14 days following vaccination. Bacterial parenteral vaccines typically don't have much response in animals less than 3 weeks of age with the exception of



*Clostridial perfringens* toxoids that have an immune response when administered at 3 days of age.

### **Calves (<3 months)**

#### Respiratory Diseases

MLV Intranasal vaccines (Depends on Maternal Antibody levels-MANY  
MLV IM or SC **NOT EFFECTIVE BEFORE 30-45 days-ONLY**  
adjuvanted MLV IM or SC)  
Branding time-Beef- MLV IM or SC- Adjuvanted; Inactivated Viral  
Vaccines??- Well adjuvanted, not affected by Maternal Antibody?

### ***Weaning-Puberty (Arrival)***

Vaccination programs are a routine practice in beef and dairy operations to protect cattle against bovine respiratory diseases (BRD). Current vaccine protocols recommend that calves be vaccinated prior to weaning or commingling, to provide protection against BRD. Unfortunately, many calves are not vaccinated prior to weaning or commingling into backgrounding lots, feedlots or pasture operations. These animals are at increased risk of viral infection and are predisposed to secondary bacterial pneumonia. However, the highly-stressed calf presents a unique problem, the vaccines may sometimes actually predispose the calves to more severe disease while on other occasions providing protection.

The time from vaccination to onset of protection can play an important role in subsequent management of newly arrived cattle against BRD viral agents i.e., bovine herpesvirus 1 (BHV-1; IBR) bovine respiratory syncytial virus (BRSV) and bovine viral diarrhea virus (BVDV). Commercially available MLV vaccines administered to non-vaccinated low-stress calves at weaning or at arrival to feed



yards will provide increased weight gains and protection to animals as early as 48 hr prior to an IBR exposure, at 5-7 days prior to a BVDV and 8 days prior to BRSV exposure.<sup>4,7</sup> This protection is due to the innate immune response, which is activated within hours after exposure to modified-live vaccines or infectious virus.

### **Frequency of vaccination**

No more than 1-2 doses of MLV or 2-3 doses of inactivated vaccines should be administered in young calves less than 4 months of age to develop good herd immunity against respiratory diseases.

Interval between doses of vaccine In all animals following vaccination there is expansion in the populations of responding T- and B-cells. However, to have a complete and mature immune response, this T- and B-cell expansion must not only stop but an active process of cell death (apoptosis) must also occur. This “waning process” allows “culling” T- or B-cells that may be poor responders or even cause autoimmunity to be removed by apoptosis. This whole process from vaccination to achieving mature immune response homeostasis takes at least 3 weeks. This fully developed mature primary response can then be boosted to get a true anamnestic secondary response. In many cases, cattle vaccine primary and booster doses are administered at two-week intervals. In young calves, this is done to provide an opportunity to make sure that the calves develop a primary response in the face of maternal immunity. The adjuvants that are used with most commercial vaccines provide superior immune development over older generation adjuvants like alum. Therefore, in most instances if primary



vaccination occurs after 3 weeks of age, booster vaccination beyond three weeks and even longer will be efficacious. The dogma that revaccination must occur within two weeks of the primary vaccination is not true and the anamnestic response will be better if we wait longer

### **Calves (>3 months)**

#### Respiratory

2-3 weeks prior weaning

MLV-1 dose

Inactivated-2 doses

Bacterial respiratory disease?

At weaning

MLV-Immune dysfunction- delay- a few days to a month

Inactivated-2 doses

Bacterial respiratory disease?

2-3 weeks post weaning

MLV-1 dose

Inactivated-2 doses

Bacterial respiratory disease?

### **Stressors and Vaccination**

There is ample evidence that both physical and psychological distress can cause dysfunction of the immune function in animals, leading to an increased incidence of infectious disease. Excess heat or cold, crowding, mixing, dehydration, weaning, calving, limit-feeding, shipping, noise, and restraint are stressors that are often associated with intensive animal production and have been shown to influence immune function in cattle. Also, social status, genetics, age and the duration of stress (chronic vs. acute) have been shown to be important in the



animal's response to stress. There is clear evidence that waiting to vaccinate at least 2 days and preferable as long as 2 weeks after the stress will result in better immunity and less sickness in that adjustment period after the stress

### **How do we vaccinate- Route and Good Nutritional Plane**

#### *Mucosal delivery vs parenteral delivery*

Mucosal delivery of vaccine either orally or intranasal is a strategy that has been used for three reasons: 1) mucosal responses occur earlier in the neonatal calf than parenteral, 2) the presence of systemic maternal antibody has little effect on generating antigenic mass necessary for developing an immune response that occurs following immunizing with a mucosal vaccine (in the face of maternal antibody-IFOMA) and 3) mucosal vaccination results in the generation of secretory IgA that is produced locally and protects mucosal surfaces where most pathogens are colonized and/or infect the host. For all vaccines, mucosal or parenteral, the critical immune reactions occur in the draining lymph node. With the right adjuvanted parenteral MLV vaccine a protective mucosal IgA response can occur IFOMA. The paradigm that only mucosal vaccines result in the immune response IFOMA and induce mucosal IgA is not true. However, the key ingredient for a parenteral MLV vaccine to induce mucosal immunity is the adjuvant. Most adjuvants can not overcome IFOMA and/or produce a mucosal IgA response. The more sophisticated oil-saponin adjuvants have this ability.

#### *Hydration and Nutrition*



One of the most critical issues in poor responses to vaccines are when animals have low water and feed intakes as a result of lack of supply, transportation, etc. The immune system requires hydration and energy for the barrier to be effective and for the immune system to actively respond and develop effective an immune response quickly including duration of immunity and memory from vaccination. The immune system is a major consumer of energy and in times of negative energy like seen in the newly weaned calf and the fresh dairy cow can be difficult times for the immune system to respond. The immune response requires energy, protein, vitamins and trace minerals. Both malnutrition and overfeeding may result in impairment of immune function and increased susceptibility to disease due to a deficiency or excess of proteins or calories, or a relative imbalance in vitamin or trace mineral content. Animals under intensive production conditions typically have a completely controlled diet. Therefore, it is very important that the diet, especially the vitamin and trace mineral content, be optimally formulated. Key vitamins and minerals for optimal immune function include vitamins A, C, E, and the B complex vitamins, copper (Cu), zinc (Zn), magnesium (Mg), manganese (Mn), iron (Fe), and selenium (Se). Of these zinc, copper and selenium are the “immune microminerals”. The balance of these constituents is especially important since excess or deficiency in one component may influence the availability or requirement for another. Zinc is involved in protein synthesis and antibody formation, cell differentiation, and enzyme formation and function. Zinc also plays a major role in skin and mucosa integrity, the first line of defense of the immune system. It is also essential for innate immune responses. Copper and manganese are directly involved with cell-mediated immunity and protein





matrix formation during the healing process. Copper has been linked with the ability of isolated neutrophils to kill yeast and bacterial infections. Selenium is an essential anti-oxidant. Manganese plays a role in facilitating the "germ-killing" function of macrophages.

### **Conclusions**

Management of the cow's and calf's immune system is not a simple process. Stressors and nutrition often compromise immunity. It is important that vaccinations be given at optimal times and that vaccination is not overused. Vaccination can never overcome poor management.

The full-length version of this paper was in the 2021 AABP Recent Graduate Proceedings- Article 6 <https://bovine-ojs-tamu.tdl.org/AABP/issue/view/180>



**ÁREA TEMÁTICA:**

# **DOENÇAS RESPIRATÓRIAS DOS BOVINOS**



## **ACURÁCIA DIAGNÓSTICA DOS SISTEMAS DE PONTUAÇÃO DE WISCONSIN E CALIFÓRNIA PARA DETECÇÃO DE DOENÇA RESPIRATÓRIA BOVINA (DRB) EM BEZERROS LEITEIROS PRÉ-DESAMADOS EM CONDIÇÕES AMBIENTAIS SUBTROPICAIS**

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A Doença Respiratória Bovina (DRB) é uma doença multifatorial que causa impactos negativos a curto e longo prazo. A detecção precoce é crucial para resposta imediata ao tratamento, bem como para diminuir o risco de mortalidade. Os sistemas de pontuação clínica foram desenvolvidos principalmente na América do Norte para triagem de bezerros doentes, e essas ferramentas também são utilizadas em países subtropicais e tropicais, como o Brasil. No entanto, não se sabe se esses sistemas de pontuação têm a mesma precisão em condições tropicais. Portanto, este estudo avaliou a acurácia de quatro métodos diferentes de sistemas de pontuação clínica, bem como a haptoglobina



sérica para diagnosticar a DRB em bezerros leiteiros Holandeses em condições subtropicais. Os exames complementares utilizados para o diagnóstico da DRB foram ultrassonografia torácica (UST, positivo se profundidade de consolidação  $\geq 1$ cm), ausculta torácica (AUSC, positivo se estertores, sibilos ou áreas silenciosas estavam presentes), escore de Wisconsin (WISC,  $\geq 2$  categorias com pontuações de  $\geq 2$ ), escore da Califórnia (CALIF, positivo se pontuação total  $\geq 5$ ). Além disso, a haptoglobina sérica (HAP) foi medida e classificada como positiva se  $\geq 15$  mg/dL. Foram incluídas no estudo novilhas entre 30 dias de idade e desmame ( $n= 482$ ) em 17 laticínios comerciais no estado de São Paulo. Modelos Bayesianos de classe latente foram usados com prioris informativas para a precisão de UST, AUSC e HAP, e prioris não informativas para a precisão de WISC e CALIF. A porcentagem de bezerros positivos para cada teste em cada fazenda variou de 0 a 56% para WISC, 11-51% CALIF, 0-72% UST, 0-32% AUSC e 0-100% HAP. A sensibilidade (Se) e especificidade (Sp) para WISC foram 77,9% (64,8-90,2) e 81,9% (76,3-88,2). Para CALIF, o Se foi de 67,1% (53,6-80,1) e Sp 79,1% (73,9-84,6). Para UST Se foi 59,8% (46,5-73,1) e Sp foi 84,8% (80,0-89,5), e para AUSC, Se foi 58,8% (41,3-79,8) e Sp foi 98,6% (95,7-99,9). O Se e Sp de HAP foi de 67,6% (55,3-78,8) e 46,7% (41,4-52,2), respectivamente. Conclui-se que o desempenho dos sistemas de pontuação foi semelhante ou melhor do que o encontrado em estudos norte-americanos, apesar de os bezerros estarem em ambiente tropical.

**Palavras-chave:** Doença Respiratória Bovina (BRD), Ultrassonografia, Escores, Haptoglobina



## **DIAGNOSTIC ACCURACY OF WISCONSIN AND CALIFORNIA SCORING SYSTEMS TO DETECT BOVINE RESPIRATORY DISEASE (BRD) IN PREWEANED DAIRY CALVES UNDER SUBTROPICAL ENVIRONMENTAL CONDITIONS**

Bovine respiratory disease (BRD) is a multifactorial disease which causes short- and long-term negative impacts. Early detection is crucial for a prompt response to therapy, as well as to decrease mortality risk. Clinical scoring systems have been developed mostly in North America for screening diseased calves in a risk population of animals, and these tools have also been applied in subtropical and tropical countries. However, it has been unknown whether these scoring systems had the same accuracy in tropical environmental conditions. Therefore, this study evaluated the accuracy of four different methods as well as serum haptoglobin to diagnose BRD in Holstein dairy calves in subtropical conditions. The tests used to diagnose BRD were thoracic ultrasound (TUS, positive if consolidation depth  $\geq 1$ cm), thoracic auscultation (AUSC, positive if crackles, wheezes or silent areas were present), Wisconsin score (WISC,  $\geq 2$  categories with scores of  $\geq 2$ ), California score (CALIF, positive if total score  $\geq 5$ ). Also, serum haptoglobin (HAP) was measured and classified as positive if  $\geq 15$  mg/dL. Heifers between 30 days of age and weaning ( $n = 482$ ) on 17 commercial dairies in São Paulo state were enrolled in this study. Bayesian latent class models were used with informative priors for the accuracy of TUS, AUSC and HAP, and non-informative priors for the accuracy of WISC and CALIF. The percentage of calves positive for each test on each farm ranged from 0 to 56% for WISC, 11-51% for



CALIF, 0-72% for TUS, 0-32% for AUSC, and 0-100% for HAP. The sensitivity (Se) (95% credible interval (CI)) and specificity (Sp) for WISC were 77.9% (64.8–90.2) and 81.9% (76.3-88.2). For CALIF, the se was 67.1% (53.6–80.1) and Sp 79.1% (73.9-84.6). For TUS Se was 59.8% (46.5–73.1) and Sp was 84.8% (80.0–89.5), and for AUSC, Se was 58.8% (41.3-79.8) and Sp was 98.6% (95.7-99.9). The Se and Sp of HAP was 67.6% (55.3-78.8) and 46.7% (41.4-52.2), respectively. The performance of the scoring systems was similar to or better than found in North American studies, despite the fact that calves were in a tropical environment.

**Keywords:** bovine respiratory disease (BRD), ultrasonography, scores, haptoglobin



## **ANÁLISE DA CORRELAÇÃO ENTRE ULTRASSONOGRAFIA E PARÂMETROS AVALIADOS NOS ESCORES DE WISCONSIN E DE CALIFÓRNIA COMO MÉTODOS DIAGNÓSTICOS PARA PNEUMONIAS EM BEZERROS**

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Sabe-se que o diagnóstico das doenças respiratórias em bezerras representa um grande desafio para a pecuária brasileira, uma vez que estas afecções possuem sinais clínicos pouco específicos em seus estágios iniciais, apresentam alta morbidade e podem culminar em perda de produtividade e morte. Assumindo a ultrassonografia torácica (UST) como método diagnóstico para pneumonias mais próximo de um padrão ouro dentre os que temos disponíveis atualmente, foi realizado um estudo transversal em uma fazenda produtora de leite em Minas Gerais com o objetivo de analisar a correlação entre a UST e os sinais clínicos avaliados nos escores de Wisconsin (WI) e de Califórnia (CA). Desta maneira, 190 bezerras holandesas de 1-180 dias de idade foram avaliadas e foi realizado o teste de correlação de postos de Spearman entre os casos positivos na UST e



os parâmetros avaliados nos escores de WI e de CA. Os resultados indicaram valor de  $r$  de Spearman de 0.385 para Tosse WI ( $p < 0.001$ ), 0.211 para Secreção Nasal WI ( $p = 0.004$ ), 0.365 para Tosse CA ( $p < 0.001$ ), 0.201 para Secreção Nasal CA ( $p = 0.005$ ), e 0.330 para Padrão Respiratório CA ( $p < 0.001$ ). Os demais parâmetros avaliados obtiveram valor de  $p > 0.05$  e por isso não foram considerados estatisticamente relevantes. Desta maneira, observou-se fraca correlação entre estes sinais clínicos e a UST, sendo tosse e secreção nasal, em ambos os escores, e padrão respiratório no escore de CA, os parâmetros com melhor correlação com UST. Considerando que, muitas vezes, os sinais clínicos não estão presentes, conclui-se que a avaliação de escores clínicos respiratórios apresenta grande limitação diagnóstica, apesar de sua relevância na rotina, dada a escassez de métodos efetivos de diagnóstico de doenças respiratórias em bovinos.

**Palavras-chave:** DRB, Tosse, Secreção Nasal, Diagnóstico, Ultrassonografia torácica





## **ANALYSIS OF THE CORRELATION BETWEEN ULTRASOUND AND PARAMETERS EVALUATED IN THE WISCONSIN AND CALIFORNIA SCORES AS METHODS FOR PNEUMONIA DIAGNOSIS IN CALVES**

It is known that the diagnosis of respiratory diseases in calves represents a major challenge for Brazilian livestock, since these conditions have little to nonspecific clinical signs in their early stages, high morbidity and can lead to productivity losses and death. Assuming thoracic ultrasound (TUS) as the closer to a gold standard among currently available calf pneumonia diagnostic methods, a cross-sectional study was carried out in a dairy farm in Minas Gerais with the objective of analyzing the correlation between UST and the clinical signs evaluated in the Wisconsin (WI) and California (CA) scores. Thus, 190 Holstein calves from 1 to 180 days of age were evaluated and the Spearman rank correlation test was performed between the positive cases in UST and the parameters evaluated in the WI and CA scores. The results indicated a Spearman rho value of 0.385 for Cough WI ( $p < 0.001$ ), 0.211 for Nasal Secretion WI ( $p = 0.004$ ), 0.365 for Cough CA ( $p < 0.001$ ), 0.201 for Nasal Secretion CA ( $p = 0.005$ ), and 0.330 for CA Breathing Pattern ( $p < 0.001$ ). Other parameters evaluated obtained a value of  $p > 0.05$  and therefore were not considered statistically relevant. Therefore, a weak correlation between clinical signs and UST was observed, with cough and nasal secretion, in both scores, and breathing pattern in the CA score, being the parameters with the best correlation with UST. Considering that clinical signs are



often not present, it is concluded that the evaluation of respiratory clinical scores has a major diagnostic limitation, despite its relevance in routine, given the scarcity of effective methods for diagnosing respiratory diseases in cattle.

**Keywords:** BRD, Cough, Nasal Discharge, Diagnosis, Thoracic Ultrasound



## **ASPECTOS ECOGRÁFICOS DE LESÕES GRANULOMATOSAS EM SETE BOVINOS COM TUBERCULOSE ATENDIDOS NA CLÍNICA DE BOVINOS DE GARANHUNS – UFRPE**

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As afecções respiratórias representam uma das principais causas de morbidade, mortalidade e perda econômica para pecuária bovina. A incidência de pneumonia granulomatosa (nodular) na clínica médica de ruminantes é preocupante, uma vez que a principal delas é a tuberculose bovina que representa um risco à saúde pública por ser transmissível ao homem e a outros animais - zoonose. A tuberculose bovina é uma doença de notificação compulsória e é imprescindível, o reconhecimento das lesões causadas pela infecção por *Mycobacterium bovis* e detecção dos infectados no plantel. Neste contexto, o exame ultrassonográfico surge como uma ferramenta auxiliar no diagnóstico da tuberculose, por permitir visualização de lesões, ainda que sutis, detectar animais subclínicos e anérgicos, além de quantificar o comprometimento pulmonar em casos mais graves. Portanto, objetivou-se contextualizar a aplicação do exame ultrassonográfico transtorácico, na rotina da clínica de ruminantes, como auxílio diagnóstico da pneumonia granulomatosa e



caracterizar os aspectos ultrassonográficos das lesões observadas em sete bovinos atendidos na Clínica de Bovinos de Garanhuns - UFRPE, no período de janeiro a dezembro de 2019. Neste período foram realizados 132 exames ultrassonográficos de tórax. Destes, sete bovinos tiveram imagens sugestivas de pneumonia tuberculosa, o que representa 5,3% de todos os casos. As lesões visualizadas tinham aspecto nodular/piogranulomatoso, de distribuição difusa nos lobos pulmonares, sendo a maior ocorrência em 8<sup>o</sup> e 7<sup>o</sup> espaços intercostais (EIC), região cranioventral, em ambos antímeros, sendo também observadas em outros órgãos, como: baço (5/7), fígado (4/7), linfonodos mesentéricos (4/7), parede ruminal/reticular (3/7 [cada]) e úbere/útero (1/7[cada]). Os nódulos apresentavam ecotextura heterogênea, hiperecogênicos, com halos hipoecóicos, medindo 1.97 cm (média), causando artefatos do tipo *tail comet*, pulmão com irregularidades pleurais, efusão pleural, padrão exacerbado de reverberações, expandindo o campo pulmonar caudo-ventralmente e com áreas de consolidação (4/7). As imagens sugeriram principalmente tuberculose miliar (5/7), sendo o sistema digestório sítio primário em um animal (1/7). A ultrassonografia torácica pode auxiliar na diferenciação da tuberculose de outras pneumonias e/ou afecções, possibilitando a indicação clínica de isolamento dos animais suspeitos, tuberculinização, realização de biópsias e decisão da viabilidade de tratamento ou condenação do animal no plantel, reduzindo as perdas econômicas do produtor.

**Palavras-chave:** Afecções respiratórias, Bovinos, Diagnóstico por imagem, Granulomas, Pneumonia



## **ECOGRAPHIC ASPECTS OF GRANULOMATOUS LESIONS IN SEVEN CATTLE WITH TUBERCULOSIS ATTENDED AT GARANHUNS BOVINE CLINIC – UFRPE**

Respiratory diseases represent one of the main causes of morbidity, mortality and economic losses for livestock. The incidence of granulomatous pneumonia (nodular) in the ruminant medical clinic is worrying, since the main one is bovine tuberculosis, which represents a risk to public health because it is transmissible to humans and other animals - zoonosis. Bovine tuberculosis is a notifiable disease and it is essential to recognize the lesions caused by *Mycobacterium bovis* infection and detect those infected in the herd. In this context, the ultrasound examination appears as an auxiliary tool in the diagnosis of tuberculosis, as it allows the visualization of lesions, albeit subtle, detecting subclinical and anergic animals, in addition to quantifying the pulmonary involvement in more severe cases. Therefore, the objective was to contextualize the application of the transthoracic ultrasound examination, in the routine of the ruminant clinic, as a diagnostic aid for granulomatous (nodular) pneumonia and to characterize the ultrasound aspects of the lesions observed in seven cattle treated at the Clínica de Bovinos de Garanhuns - UFRPE, from January to December 2019. During this period, 132 chest ultrasound examinations were performed. Of these, seven cattle had images suggestive of tuberculous pneumonia, representing 5.3% of all cases. The lesions visualized had a nodular/pyogranulomatous aspect, with a diffuse distribution in the lung lobes,



with the highest occurrence in the 8th and 7th intercostal spaces (ICE), cranioventral region, in both antimeres, being also observed in other organs, such as: spleen (5/ 7), liver (4/7), mesenteric lymph nodes (4/7), rumen/reticular wall (3/7 [each]) and udder/uterus (1/7 [each]). The nodules had a heterogeneous, hyperechoic echotexture, with hypoechoic halos, measuring 1.97 cm (mean), causing tail comet-like artifacts, lung with pleural irregularities, pleural effusion, exacerbated pattern of reverberations, expanding the lung field caudoventrally and with areas of consolidation (4/7). The images mainly suggested miliary tuberculosis (5/7), with the digestive system being the primary site in one animal (1/7). Thoracic ultrasound can help to differentiate tuberculosis from other pneumonias and/or conditions, allowing the clinical indication of isolation of suspected animals, tuberculinization, performing biopsies and deciding on the feasibility of treatment or condemnation of the animal in the herd, reducing the economic losses of the animal. producer.

**Keywords:** Respiratory disorders, Cattle, Diagnostic imaging, Granulomas, Pneumonia



## **ANÁLISE DE CONCORDÂNCIA ENTRE MÉTODOS PARA DIAGNÓSTICO DE DOENÇA RESPIRATÓRIA EM 190 BEZERRAS DA RAÇA HOLANDESA UM ESTUDO TRANSVERSAL**

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Entre as principais causas de mortalidade em bezerras o complexo doença respiratória bovina (DRB) tem como desafio o diagnóstico tardio e equivocado que é feito a campo. O estudo foi realizado com 190 bezerras da raça Holandesa com idade entre 7 a 180 dias de uma fazenda de produção leiteira comercial no Estado de Minas Gerais. Todos os animais foram submetidos a avaliação única: Ultrassonografia Torácica (UST), Escore de Wisconsin (WI), Escore da Califórnia (CA) e Auscultação Pulmonar (AP) por avaliador único. Realizou-se análise de concordância entre métodos através do índice Kappa. A UST teve concordância razoável com os escores de WI ( $k = 0,302$ ) e da CA ( $k = 0,274$ ) e moderada com a AP ( $k = 0,405$ ). Houve concordância moderada entre os escores clínicos respiratórios de WI e da CA ( $k = 0,485$ ). Houve concordância mínima ( $k=0,188$ ) e moderada ( $k=0,316$ ) entre a AP e os escores da CA e de WI respectivamente.



A UST permite a identificação e mensuração de áreas de consolidação pulmonar que podem se enquadrar em casos iniciais, subclínicos além de lesões crônicas. A correlação moderada entre a AP e a UST demonstra o nível de correlação entre a técnica semiológica e a UST na constatação de áreas acometidas devido ao fato de ambas avaliarem focos de pneumonia. A ausência de correlações mais significativas entre os escores clínicos e a UST sugere que os métodos podem estar identificando alterações diferentes dado que os scores clínicos além de possuir parâmetros relacionados a pneumonia avaliam possíveis alterações no trato respiratório superior. Lesões crônicas podem também não aparecer nos scores clínicos, mas se apresentam no ultrassom, dentro de um estudo transversal não é possível qualificar as lesões. Tradando-se de propostas similares, esperava-se concordância elevada entre os escores clínicos de WI e CA porém a diferença na graduação dos parâmetros dos scores podem ter interferido nesta relação. Os métodos possuem abrangências diferentes e se correlacionam com intensidades diferentes. A ultrassonografia pulmonar é importante para diagnóstico de pneumonias, porém deve estar associada ao uso de escores clínicos para avaliação completa do trato respiratório dos bezerros.

**Palavras-chave:** Ultrassonografia, pneumonia, ausculta, escores





## **ANALYSIS OF AGREEMENT BETWEEN METHODS FOR RESPIRATORY DISEASE DIAGNOSIS IN 190 HOLSTEIN HEIFERS A CROSS-SECTIONAL STUDY**

Among the main causes of mortality in calves, the bovine respiratory disease complex (RBD) has the challenge of late and mistaken diagnosis done in the field. The study was conducted with 190 Holstein heifers aged between 7 and 180 days from a commercial dairy farm in the State of Minas Gerais. All animals underwent a single evaluation: Thoracic Ultrasonography (UST), Wisconsin Score (WI), California Score (CA) and Pulmonary Auscultation (AP) by a single evaluator. Analysis of agreement between methods was performed using the Kappa index. UST had reasonable agreement with the scores of WI ( $k = 0.302$ ) and AC ( $k = 0.274$ ) and moderate agreement with AP ( $k = 0.405$ ). There was moderate agreement between WI and AC respiratory clinical scores ( $k = 0.485$ ). There was minimal ( $k=0.188$ ) and moderate ( $k=0.316$ ) agreement between AP and AC and WI scores, respectively. The UST allows the identification and measurement of areas of pulmonary consolidation that can be classified in initial and subclinical cases, in addition to chronic lesions. The moderate correlation between AP and UST demonstrates the level of correlation between the semiological technique and UST in finding affected areas due to the fact that both assess foci of pneumonia. The absence of more significant correlations between the clinical scores and the UST suggests that the methods may be identifying different alterations, given that the clinical scores, in addition to having parameters related to pneumonia, evaluate possible alterations in the upper respiratory tract. Chronic



lesions may also not appear on clinical scores, but they could appear on ultrasound, within a cross-sectional study it is not possible to qualify the lesions. With similar proposals, a high agreement was expected between the clinical scores of WI and CA, but the difference in the grading of the score parameters may have interfered in this relationship. The methods have different scopes and correlate with different intensities. Pulmonary ultrasound is important for diagnosing pneumonia, but it should be associated with the use of clinical scores for a complete assessment of the respiratory tract of calves.

**Key words:** Ultrasonography, pneumonia, auscultation, scores



## AVALIAÇÃO DA EFICIÊNCIA NO DIAGNÓSTICO DE ENFERMIDADES RESPIRATÓRIAS – DADOS PRELIMINARES

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O Brasil possui 218,2 milhões de cabeças bovinas e chegou a produzir 35,4 bilhões de litros de leite em 2020. Para o sistema se manter economicamente viável é importante ser mantido o alto desempenho produtivo. Neste contexto, a sanidade das futuras produtoras é fundamental na fase inicial pré-púbere. Estima-se que 75% das bezerras venham a óbito no primeiro ano de vida e uma das causas são as doenças respiratórias. Diante disto, o objetivo deste estudo foi avaliar qual a forma de diagnosticar enfermidades respiratórias é mais precisa. Este estudo foi realizado no mês de setembro de 2022 em uma propriedade leiteira localizada no município Salto do Lontra no Estado do Paraná. Foram utilizadas 25 fêmeas de três a cinco meses de idade, da raça holandesa, com histórico de doença respiratória e mantidas em bezerreiros coletivos. Estas foram avaliadas pela auscultação pulmonar e os animais que apresentaram alterações, foram descritos como positivos para doença



respiratória. Estes animais também foram avaliados pelo escore respiratório de *Wisconsin* (foram atribuídos escores de zero a três baseados na interpretação dos sinais clínicos: 0 normal, 1 leve, 2 moderado e 3 grave). Quando o bovino atingiu uma pontuação igual ou superior a cinco é considerado positivo. Por fim, a avaliação ultrassonográfica pulmonar (padrão ouro) foi realizada com sonda micro convexa de 6.5 MHz (10 pontos tricotomizados por lado da região pulmonar). As alterações observadas foram: ausência de linhas A, presença de linhas B, consolidação pulmonar, presença de líquido pulmonar e alteração no padrão de ecogenicidade (as bezerras com todos os critérios presentes são positivas). Os resultados obtidos foram avaliados pelo Teste de Fisher ( $p < 0,05$ ) pelo software estatístico Graphpad Prism 9.0. Como resultado, a ultrassonografia pulmonar (grupo controle) quando comparado com a auscultação não apresentou associação ( $p = 0,0639$ ) demonstrando sua baixa eficiência. O escore respiratório *Wisconsin*, comparado com o exame ultrassonográfico, apresentou associação ( $p = 0,0365$ ). Pode-se concluir que o escore respiratório demonstrou ser efetivo para o diagnóstico precoce principalmente na ausência de equipamento ultrassonográfico. Já o método de auscultação se demonstrou um método ultrapassado quando trata-se de diagnóstico precoce de doenças respiratórias a campo.

**Palavras-chave:** Bovinos. Doenças respiratórias. Precocidade. Ultrassom



## EVALUATION OF EFFICIENCY IN THE DIAGNOSIS OF RESPIRATORY DISEASES - PRELIMINARY DATA

Brazil has 218.2 million head of cattle and came to produce 35.4 billion liters of milk in 2020. For the system to remain economically viable it is important to maintain high productive performance. In this context, the health of future producers is fundamental in the initial pre-pubertal phase. It is estimated that 75% of the calves die in the first year of life and one of the causes are respiratory diseases. Therefore, the objective of this study was to evaluate which is the most accurate way to diagnose respiratory diseases. This study was conducted in September, 2022, in a dairy farm located in the city of Salto do Lontra, Paraná State. Were used 25 Holstein females, three to five month old, with a history of respiratory disease and kept in collective calf houses. They were evaluated by lung auscultation and the animals that presented alterations were described as positive for respiratory disease. These animals were also evaluated by the *Wisconsin* Respiratory Score (scores from zero to three were assigned based on the interpretation of clinical signs: 0 normal, 1 mild, 2 moderate and 3 severe). When the bovine reached a score of five or more it is considered positive. Finally, the pulmonary ultrasonographic evaluation (gold standard) was performed with a 6.5 MHz micro convex probe (10 trichotomized points per side of the lung region). The changes observed were: absence of A lines, presence of B lines, lung consolidation, presence of pulmonary fluid and altered echogenicity pattern (calves with all criteria present are positive). The results obtained were evaluated



by Fisher's Test ( $p < 0.05$ ) using Graphpad Prism 9.0 statistical software. As a result, lung ultrasonography (control group) when compared to auscultation showed no association ( $p = 0.0639$ ) demonstrating its low efficiency. The Wisconsin Respiratory Score, compared to the ultrasonography, presented an association ( $p = 0.0365$ ). It can be concluded that the respiratory score proved to be effective for early diagnosis, especially in the absence of ultrasonography equipment. The auscultation method, on the other hand, has proven to be an outdated method when it comes to early diagnosis of respiratory diseases in the field.

**Keywords:** Cattle. Precocity. Respiratory diseases. Ultrasound



## **AVALIAÇÃO DA RESPOSTA SOROLÓGICA INDUZIDA POR VACINAS REPRODUTIVAS VIVAS MODIFICADAS CONTRA O HERPESVÍRUS BOVINO TIPO 1 (BOHV-1) EM NOVILHAS GIROLANDO**

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Esta pesquisa avaliou a eficácia de vacinas vivas modificadas contra o BoHV-1. Novilhas Girolando (n=29), entre 6 e 10 meses de idade, com títulos de anticorpos basais contra BoHV-1 (Cooper)  $\leq 4,0$ . As novilhas foram divididas em 4 grupos, de acordo com a formulação: Vacina A (n=9), polivalente contendo BoHV-1 vivo modificado (cepa Passagem C-13), dose única; Vacina B (n=7), polivalente, contendo BoHV-1 modificado ao vivo e termossensível, diluída em



adjuvante tipo ISCOM, 2 doses; Vacina C (n=7), monovalente, contendo BoHV-1 vivo com dupla deleção dos genes gE- e tk- tipo 1, cepa CEDDEL, dose única; Grupo controle D (n=6). Amostras de sangue foram coletadas na primovacinação (D0), e aos 30 e 60 dias após a 1ª dose. Anticorpos específicos (ACs) foram medidos por neutralização de vírus e ELISA sanduíche. Os dados foram parametrizados e analisados pelo PROC-MIXED e as variáveis qualitativas pelo qui-quadrado. A vacina B foi a que induziu os maiores títulos de ACs neutralizantes no D60 ( $210,28 \pm 57,09$ ), seguida pelas vacinas A ( $74,66 \pm 14,11$ ) e C ( $36,57 \pm 7,58$ ), respectivamente. Títulos de proteção ( $\geq 32$ ) foram detectados em 100% (9/9), 100% (7/7) e 57,2% (4/7) das novilhas A, B e C, respectivamente. No ELISA, a vacina B induziu maiores títulos de ACs totais ( $2011,43 \pm 354,10$ ), e as vacinas A ( $1440,00 \pm 357,77$ ) e C ( $1188,57 \pm 354,10$ ) apresentaram resultados semelhantes. Todos os animais vacinados soroconverteram e apresentaram um aumento de 4x em relação aos títulos basais. De acordo com os resultados, desde que seguidas as recomendações dos fabricantes, em geral, a imunogenicidade do BoHV-1 na maioria das vacinas vivas e modificadas contra o BoHV-1 foi consistente.

**Palavras-chave:** vacinação; gado leiteiro; imunogenicidade; anticorpos





## **EVALUATION OF THE SEROLOGICAL RESPONSE INDUCED BY MODIFIED LIVE REPRODUCTIVE VACCINES AGAINST BOVINE HERPESVIRUS TYPE 1 (BOHV-1) IN GIROLANDO HEIFERS**

This research evaluated the efficacy of modified live vaccines against BoHV-1. Girolando heifers (n=29), between 6 and 10 months of age, with baseline antibody titers against BoHV-1 (Cooper)  $\leq 4.0$ . Heifers were divided into 4 groups, according to the formulation: Vaccine A (n=9), polyvalent containing live-modified BoHV-1 (Passage C-13 strain), single dose; Vaccine B (n=7), polyvalent, containing live-modified and thermosensitive BoHV-1, diluted in ISCOM-type adjuvant, 2 doses; Vaccine C (n=7), monovalent, containing live BoHV-1 with double deletion of the gE- and tk- type 1 genes, CEDDEL strain, single dose; Control group D (n=6). Blood samples were collected at primary vaccination (D0), and at 30 and 60 days after the 1st dose. Specific antibodies (ACs) were measured by virus neutralization and sandwich ELISA. Data were parameterized and analyzed by PROC-MIXED, and qualitative variables by chi-square. Vaccine B was the one that induced the highest titers of neutralizing ACs on D60 ( $210.28 \pm 57.09$ ), followed by vaccines A ( $74.66 \pm 14.11$ ) and C ( $36.57 \pm 7.58$ ), respectively. Protective titers ( $\geq 32$ ) were detected in 100% (9/9), 100% (7/7), and 57.2% (4/7) of heifers A, B, and C, respectively. In the ELISA, vaccine B induced higher titers of total ACs ( $2011.43 \pm 354.10$ ), and vaccines A ( $1440.00 \pm 357.77$ )



and C ( $1188.57 \pm 354.10$ ) showed similar results. All vaccinated animals seroconverted and showed a 4x increase in relation to baseline titers. According to the results, as long as the manufacturers' recommendations were followed, in general, the immunogenicity of BoHV-1 in most live and modified vaccines against BoHV-1 was consistent.

**Keywords:** vaccination; dairy cattle; immunogenicity; antibodies



## AVALIAÇÃO DE UMA VACINA DIRECIONADA A SUBUNIDADE CONTRA BVDV EM BOVINOS DE CORTE

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A doença respiratória bovina (BRD) é um grave problema sanitário e econômico na indústria da carne bovina, muitas vezes associada ao transporte e causada por diferentes patógenos. Neste estudo, avaliamos o efeito de uma nova vacina direcionada a subunidades contra o Vírus da Diarreia Viral Bovina (BVDV) em bovinos confinados, um dos principais agentes virais da BRD. O desempenho da nova vacina foi avaliado em um total de 2.992 animais (grupo controle, N=1.491 e grupo de tratamento, N=1.501). Os animais de ambos os grupos receberam o plano sanitário de rotina: duas doses das vacinas clostridial, respiratória e antirrábica. Os animais do grupo de tratamento também receberam duas doses de uma vacina de subunidade direcionada contra BVDV (Vedevax Block®). A circulação viral (P80 Elisa) e a resposta imune (E2 Elisa) foram avaliadas, bem como casos de BRD. O grupo Controle teve um número maior de animais positivos para anticorpos anti-P80 e significativamente menos animais positivos para anticorpos anti-E2 em comparação com o grupo Tratamento (69% vs 61%



e 71% vs 99%, respectivamente,  $p=0,003$ ), consistente com a circulação viral natural dentro deste grupo. O grupo Controle também teve significativamente mais animais tratados para casos de BRD em comparação ao grupo Tratamento (5,9% vs 3,7%,  $p=0,02$ ). Esses resultados são consistentes com uma resposta robusta de anticorpos induzida por vacina e uma redução da circulação de BVDV dentro desse grupo, levando a um número menor de animais que precisam ser tratados para BRD.

**Palavras-chave:** Bovinos de corte, BVD, vacinação



## EVALUATION OF A SUBUNIT TARGETED VACCINE AGAINST BVDV IN FEEDLOT CATTLE

Bovine respiratory disease (BRD) is a serious health and economic problem in the beef industry, which is often associated with transportation and caused by different pathogens. In this study we evaluated the effect of a novel subunit targeted vaccine against Bovine Viral Diarrhea Virus (BVDV) on feedlot cattle, a major viral agent of BRD. The performance of the novel vaccine was evaluated in a total of 2992 animals (Control group, N=1491 and Treatment group, N=1501). Animals of both groups received the routine sanitary plan: two doses of clostridial, respiratory and rabies vaccines. Animals within the treatment group also received two doses of a targeted subunit vaccine against BVDV (Vedevax Block<sup>®</sup>). Viral circulation (P80 Elisa) and immune response (E2 Elisa) were evaluated as well as BRD cases. The Control group had a greater number of animals positive for anti-P80 antibodies and significant fewer animals positive for anti-E2 antibodies compared to the Treatment group (69% vs 61% and 71% vs 99%, respectively,  $p=0,003$ ), consistent with natural viral circulation within this group. The Control group had also significant more animals treated for BRD cases compared to the Treatment group (5,9% vs 3,7%,  $p=0,02$ ). These results are consistent with a robust vaccine-induced antibody response and a reduction of the BVDV circulation within this group leading to a fewer number of animals needing to be treated for BRD.

**Keywords:** Feedlot cattle, BVDV, Vaccination



## **AVALIAÇÃO DO POTENCIAL PROTETIVO DAS VACINAS CONTRA O HERPESVÍRUS BOVINO TIPO 1 (BoHV-1) EM MODELO *GUINEA PIG***

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Herpesvírus Bovino tipo 1 (BoHV-1) causa significativas perdas econômicas nos rebanhos mundiais, por ser um vírus associado ao Complexo Respiratório Bovino e doenças reprodutivas. O objetivo do trabalho foi verificar o perfil imunogênico de sete vacinas contendo o BoHV-1, por meio da mensuração dos títulos de anticorpos específicos utilizando a virusneutralização e ELISA. O projeto foi aprovado pela CEUA/FMVZ- USP, protocolada sob o nº 7782291020. Foram utilizadas 56 cobaias (*Guinea pig*) da raça Hartley, 30 dias de idade, distribuídas em grupos A-G, além do controle não vacinado. As cepas de BoHV-1 e adjuvantes dos produtos foram: vacina A (n= 6) polivalente, BoHV inativado em hidróxido de alumínio (Selenato de sódio e timerosal); vacina B (n=6) polivalente, BoHV-1 vivo com dupla deleção nos genes gE-tK- Tipo 1, cepa (CEDDEL); vacina C (n=7) polivalente, BoHV-1 inativado em hidróxido de alumínio; vacina D (n=6) polivalente, cepas alteradas termossensíveis BoHV-1 em Immunostimulation Complexes (ISCOMs). Vacina E (n=6), polivalente,



BoHV-1 e 5 inativados em hidróxido de alumínio à 10% e 10 mg/dose de selênio; vacina F (n=6) polivalente; BoHV-1 inativado (*Los Angeles*); vacina G (n=6) polivalente, BoHV-1 e 5 inativados em Pilatus GHA500 aquoso; e grupo controle (n=7). As cobaias receberam duas doses das vacinas (intervalo de 21 dias), por via subcutânea, sendo o volume 1/5 da dose indicada para bovinos. A coleta foi feita no momento da primovacinação e após 10 dias da revacinação. Os títulos de Acs foram determinados por virusneutralização e ELISA. Os dados foram parametrizados e os efeitos do grupo, tempo e interação grupo\*tempo foi realizada pelo PROC-MIXED (Statistical Analyses System versão 9.4). Foi detectado efeito do grupo ( $P \leq 0,0001$ ), tempo ( $P=0,0017$ ) e interação grupo\*tempo ( $P \leq 0,0001$ ). Todas as cobaias apresentaram títulos de anticorpos neutralizantes  $\leq 4,0$  na primovacinação. Os maiores títulos de anticorpos neutralizantes após as vacinações foram observados nas cobaias vacinadas com as formulações B e D. Os títulos de anticorpos totais mensurados pelo ELISA também foram maiores nos animais vacinados com as formulações B e D, seguidas da vacina C. As vacinas que possuíam BoHV-1 vivos e modificados na sua formulação apresentaram melhor resposta sorológica.

**Palavras chaves:** Anticorpos, virusneutralização, ELISA



## EVALUATION OF THE PROTECTIVE POTENTIAL OF VACCINES AGAINST BOVINE HERPES VIRUS TYPE 1 (BoHV-1) IN A GUINEA PIG MODEL

Bovine Herpes Virus type 1 (BoHV-1) causes significant economic losses in herds worldwide, as it is a virus associated with the Bovine Respiratory Complex and reproductive diseases. The objective of this work was to verify the immunogenic profile of seven vaccines containing BoHV-1, by measuring the titers of specific antibodies using virus neutralization and ELISA. The project was approved by CEUA/FMVZ-USP, registered under n<sup>o</sup>. 7782291020. Fifty-six *guinea pigs* of the Hartley breed, 30 days old, were distributed in groups A-G, in addition to the non-vaccinated control. The BoHV-1 strains and product adjuvants were: vaccine A (n=6) polyvalent, inactivated BoHV, diluted in aluminum hydroxide (Sodium selenate and thimerosal), vaccine B (n=6) polyvalent, live BoHV-1 with double deletion in gE-tK-Type 1 genes, strain (*CEDDEL*); vaccine C (n=7) polyvalent, inactivated BoHV-1 in aluminum hydroxide; vaccine D (n=6) polyvalent, altered thermosensitive strains BoHV-1 in Immunostimulation Complexes (ISCOMs). Vaccine E (n=6), polyvalent, BoHV-1 and 5 inactivated in aluminum hydroxide at 10% and 10 mg/dose of selenium; F vaccine (n=6) polyvalent, inactivated BoHV-1 (*Los Angeles*); vaccine G (n=6) polyvalent, BoHV-1 and 5 inactivated in aqueous Pilatus GHA500; and control group (n=7). The guinea pigs received two doses of vaccines (21-day interval), subcutaneously,





the volume being 1/5 of the dose indicated for cattle. Collection was performed on the primovaccination and 10 days after revaccination. Abs titers were determined by virus neutralization and ELISA. The data were parameterized and the effects of group, time and group\*time interactions were performed by PROC-MIXED (Statistical Analyses System versão 9.4). It was detected effect of group ( $P \leq 0.0001$ ), time ( $P=0.0017$ ) and interaction group\*time ( $P \leq 0.0001$ ). All Foi detectado efeito do grupo ( $P \leq 0,0001$ ), tempo ( $P=0,0017$ ) e interação grupo\*tempo ( $P \leq 0,0001$ ). Todas as cobaias apresentaram títulos de anticorpos neutralizantes  $\leq 4,0$  no início do estudo (dia 1). Os maiores títulos de anticorpos neutralizantes ao final da pesquisa (dia 31) foram observados nas cobaias vacinadas com as formulações B and D. Os títulos de anticorpos totais mensurados pelo ELISA também foram maiores nos animais vacinados com as formulações B e D, seguidas da vacina C. Guinea pig presented neutralizing antibodies titers less than 4.0 on primovaccination time. Highest titers of neutralizing antibodies after booster was observed in animals vaccinated with formulation B and D. The total antibodies measured by ELISA also was higher in animals vaccinated with formulations B and D, followed by vaccine C. Vaccines composed of live-modified BoHV-1 in their formulation showed greater serological response.

**Keyword:** Antibody, virusneutralization, ELISA



## CARACTERÍSTICAS DO APARELHO RESPIRATÓRIO EM BEZERROS DURANTE QUADRO PNEUMÔNICO DE MANHEMIOSE POR BRONCOSCOPIA

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A *Mannheimia haemolytica* está relacionada ao Complexo Doença Respiratória dos Bovinos, a qual se atribui evolução para uma forma grave de pneumonia fibrinonecrótica. Atualmente na literatura, existem poucas evidências científicas a respeito de avaliações do quadro evolutivo de manheimiose. Assim, objetivou-se avaliar as alterações no aparelho respiratório de bezerros no processo de evolução da doença. Para tal, foram utilizados 12 bezerros machos, hípidos, que foram infectados com *M. haemolytica*, com dose de 5 mL contendo  $1 \times 10^9$  unidades formadoras de colônias (UFC), infundida na luz da traqueia. Os animais foram distribuídos aleatoriamente, em dois grupos, seis cada: Grupo 1 (G1): tratamento com norfloxacina e flunixinina meglumina; Grupo 2 (G2): tratamento com norfloxacina. O lavado bronco alveolar (LBA) foi realizado por endoscopia em quatro momentos: Momento 1 (M1): dois dias pré-infecção; Momento 2 (M2): pós-infecção e aparecimento dos sintomas; Momento 3 (M3): um dia pós-tratamento; Momento 4 (M4): sete dias pós-tratamento. Os animais



foram avaliados por broncoscopia e isolamento do agente. Utilizou-se o animal sadio anteriormente às manifestações clínicas da mannheimiose como controle de si mesmo. Na avaliação dos resultados foi possível inferir que no exame de broncoscopia, havia características normais no aparelho respiratório no M1. Já em M2 identificou-se alterações características de quadro pneumônico, no qual os bezerros, apresentaram grande quantidade de secreção fibrinonecrótica, de coloração que alternou do amarelo até o esverdeado, e com vasos ingurgitados e bem evidentes. Em M3, essas alterações amenizaram e duas semanas após, os animais voltaram à sua condição inicial. Ainda, foi encontrada *M. haemolytica* em 100 % das amostras de *swab* nasal no M2. Somado a isso, a norfloxacin se mostrou eficaz ao tratamento como observado na regressão do quadro pneumônico após o tratamento e ausência de re-isolamento de *M. haemolytica* na cultura bacteriológica, no M3 e M4. Desta forma, conclui-se que a mannheimiose possui rápida evolução, chegando a ocasionar pneumonia fibrinonecrótica . Contudo, o tratamento com norfloxacin se mostrou eficaz no controle da infecção e manutenção do *status* de saúde dos animais.

**Palavras-chave:** Afecções respiratórias, Bovinocultura leiteira, Broncoscopia, Pneumonia fibrinonecrótica



## CHARACTERISTICS OF THE RESPIRATORY SYSTEM IN CALVES DURING PNEUMONIC MANHEMIOSIS BY BRONCHOSCOPY

*Mannheimia haemolytica* is related to the Bovine Respiratory Disease Complex, which evolves into a severe form of fibrinonecrotic pneumonia. Currently in the literature, there is little scientific evidence regarding evaluations of the evolutionary picture of mannheimiosis. Thus, the objective was to evaluate the changes in the respiratory system of calves in the process of disease evolution. For this, 12 healthy male calves were used, which were infected with *M. haemolytica*, with a dose of 5 mL containing  $1 \times 10^9$  colony forming units (CFU), infused in the tracheal lumen. The animals were randomly divided into two groups, six each: Group 1 (G1): treatment with norfloxacin and flunixin meglumine; Group 2 (G2): treatment with norfloxacin. Bronchoalveolar lavage (BAL) was performed by endoscopy at four moments: Moment 1 (M1): two days pre-infection; Moment 2 (M2): post-infection and onset of symptoms; Moment 3 (M3): one day post-treatment; Moment 4 (M4): seven days post-treatment. The animals were evaluated by bronchoscopy and agent isolation. The healthy animal before the clinical manifestations of mannheimiosis was used as self-control. In the evaluation of the results, it was possible to infer that in the bronchoscopy exam, there were normal characteristics in the respiratory system in M1. In M2, alterations characteristic of the pneumonic condition were identified, in which the calves presented a large amount of fibrinonecrotic secretion, with a color that varied from yellow to greenish, and with engorged and very evident vessels. In



M3, these changes eased and two weeks later, the animals returned to their initial condition. Furthermore, *M. haemolytica* was found in 100% of nasal swab samples in M2. In addition, norfloxacin proved to be effective in the treatment, as observed in the regression of the pneumonic condition after treatment and the absence of re-isolation of *M. haemolytica* in the bacteriological culture, in M3 and M4. Thus, it is concluded that mannheimiosis has a rapid evolution, reaching the point of causing fibrinonecrotic pneumonia. However, treatment with norfloxacin was effective in controlling the infection and maintaining the health status of the animals.

**Keywords:** Respiratory disorders, Dairy cattle, Bronchoscopy, Fibrinonecrotic Pneumonia



## **CARCINOMA PULMONAR ADENOESCAMOSO EM BOVINO - RELATO DE CASO**

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Carcinoma adenoescamoso é uma neoplasia maligna, composta por um componente glandular e um componente de células espinhosas. É raro na medicina veterinária e principalmente na clínica de bovinos, sem etiologia pré-estabelecida. O presente trabalho busca relatar um caso de carcinoma pulmonar adenoescamoso em um bovino. Foi atendida na Clínica de Bovinos e Pequenos Ruminantes da Universidade de São Paulo, uma fêmea, bovina, SRD, 7 anos de idade, oriunda de propriedade com criação extensiva, em Micaratu-SP. Possuía histórico de emagrecimento progressivo há aproximadamente dois meses, com tosse esporádica. Na admissão, apresentava apatia, escore de condição corporal 1,5, desidratação (6%), aumento de linfonodos cervicais superficiais e mandibulares, secreção nasal serosa bilateral, sensibilidade à percussão pulmonar, crepitação pulmonar grossa bilateral, mais intensa no lado direito, e atonia ruminal. Os exames complementares indicaram discreta leucopenia (4.000/uL) e hipoalbuminemia (1,96mg/dL). Na ultrassonografia pulmonar,



evidenciaram-se áreas circulares anecóicas (~1,5cm) sugestivas de nódulos em pulmão e pleura visceral. Na endoscopia traqueobrônquica notou-se coágulo em seio nasal. Por meio da clínica apresentada e achados ultrassonográficos elencou-se a tuberculose como diagnóstico presuntivo e amostra de lavado traqueal foi coletada para cultura, identificando-se micobacterias não tuberculosas. Iniciou-se tratamento com enrofloxacina, meloxicam, bromexina e dipirona. Após tratamento prolongado, apresentando melhora pouco expressiva e sem evolução positiva, decidiu-se pela eutanásia. Durante a necropsia, realizada pelo Serviço de Patologia Animal da Universidade de São Paulo, foram observadas formações nodulares pedunculadas de 0,5 a 5,0 cm de diâmetro e coloração amarelo-acastanhada na musculatura intercostal, diafragma, superfície pulmonar, região periportal, na região de glote à base do crânio e em múltiplos linfonodos, sendo estabelecido, por meio da histopatologia, o diagnóstico de carcinoma pulmonar adenoescamoso com metástase. Há relato de adenocarcinoma pulmonar associado à infecção por *Mycobacterium* sp. em bovino, porém não há, na literatura consultada, casos de carcinoma adenoescamoso, não sendo possível prever a correlação entre a infecção por micobacterias não tuberculosas com o neoplasma, ou determinar a causa da mesma. Conclui-se que o carcinoma pulmonar adenoescamoso, deve, portanto, ser considerado como diagnóstico diferencial em doenças pulmonares crônicas, e mais estudos são necessários sobre a etiologia da enfermidade.

**Palavras-chave:** bovinos; neoplasia; pneumopatia



## **ADENOID-SQUAMOUS PULMONARY CARCINOMA IN CATTLE - CASE REPORT**

Adeno-squamous cell carcinoma is a malignant neoplasm, composed of a glandular component and a component of spinous cells. It's rare in veterinary medicine and especially in the cattle clinical, with no pre-established etiology. The present work seeks to report a case of adeno-squamous lung carcinoma in a bovine. A female bovine, crossbred, 7 years old, from a property with extensive breeding, in Micaratu-SP, was attended at Cattle and Small Ruminant Clinic of the University of São Paulo. The bovine had a history of progressive weight loss for approximately two months, with sporadic coughing. At admission, he presented apathy, body condition score 1.5, dehydration (6%), enlarged prescapular and submandibular lymph nodes, bilateral serous nasal discharge, pain to lung percussion, bilateral coarse lung crackling, more intense on the right side, and rumen atony. Complementary exams indicated mild leukopenia (4.000/uL) and hypoalbuminemia (1,96mg/dL). Pulmonary ultrasonography revealed circular anechoic areas (~1.5cm) suggestive of nodules in the lung and visceral pleura. Tracheobronchial endoscopy revealed a clot at nasal sinus. Based on the clinical presentation and sonographic findings, bovine tuberculosis was considered as presumptive diagnosis, and a sample of tracheal lavage was collected for culture, identifying non-tuberculous mycobacteria. The patient was treated with enrofloxacin, meloxicam, bromhexine and dipyrone. After prolonged





treatment, with little improvement and no positive evolution, was decided to euthanize the animal. During the necropsy, carried out by the Animal Pathology Department of the University of São Paulo, in which pedunculated nodular formations of 0.5 to 5.0 cm in diameter and yellow-brownish coloration were observed in the intercostal muscles, diaphragm, lung surface, periportal region, in the region from the glottis to the base of the skull and in multiple lymph nodes. There are reports of lung adenocarcinoma associated with infection by *Mycobacterium* sp. in cattle, but there aren't cases of adeno-squamous carcinoma in the consulted literature, and it isn't possible to predict the correlation between infection by nontuberculous mycobacteria and the neoplasm, or to determine its cause. We conclude that adeno-squamous cell lung carcinoma should be considered as a differential diagnosis in chronic lung diseases, and more studies are needed about the etiology of the disease.

**Keywords:** bovines; neoplasm; pneumopathy



## COMPARAÇÃO ENTRE O ESCORE DE AVALIAÇÃO CLÍNICA DA CALIFÓRNIA E A RADIOGRAFIA TORÁCICA NO DIAGNÓSTICO DA DOENÇA RESPIRATÓRIA DE BEZERROS

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A produção mundial de bovinos está concentrada em alguns países específicos, o Brasil é o segundo maior criador e com as maiores perspectivas de expansão, projetadas para mais de 53 milhões de cabeças de bezerros no ano de 2022. Os bezerros adoecem principalmente por falhas no manejo, que vão decorrer em diarreias, onfalites e as doenças respiratórias que são subdiagnosticadas dentro das propriedades. Objetivou-se analisar a técnica diagnóstica de avaliação clínica para doença respiratória do Escore da Califórnia verificando sua efetividade através da comparação com os achados na radiografia torácica. Foram avaliados 17 bezerros entre 1 e 150 dias de vida, com queixa de doença respiratória, admitidos entre agosto de 2021 e setembro de 2022 no Centro de Desenvolvimento da Pecuária da Universidade Federal da Bahia, localizado em Santo Amaro, estado da Bahia. Os pacientes foram examinados e os sinais clínicos temperatura retal, tosse, secreção nasal e ocular, posição de cabeça e orelhas e o padrão respiratório anormal foram pontuados de 0 a 5 para cada sinal de acordo com o escore clínico da Califórnia



descrito por Love et al. 2014. A radiografia torácica em decúbito latero-lateral direito e esquerdo foi o exame referência para este estudo, com os valores ajustados em 2,5 mAs e 100 Kv. Diante dos resultados evidenciou-se que 13/17 (76,4%) dos bezerros foram positivos para doença respiratória de acordo com o escore clínico da Califórnia. Já na radiografia torácica comprovou-se que 100% dos animais avaliados apresentavam alteração no padrão pulmonar, sendo então considerados como positivos para doença respiratória. O escore clínico da Califórnia mostrou boa eficiência em detectar os animais doentes com pontuações de presença ou ausência dos sinais clínicos de forma direta. Os animais que não pontuaram suficientemente para serem incluídos como positivos podem ser explicados pelo padrão de imagem radiográfica do tipo intersticial, que comprova sinais leves de acometimento pulmonar. A avaliação através do escore clínico da Califórnia, provada pelo exame radiográfico, pode ser considerada uma alternativa diagnóstica para uso nos hospitais veterinários e fazendas, auxiliando no diagnóstico precoce e na tomada de decisão quanto a tratamento e manejo preventivo.

**Palavras-chave:** Bovinos; Califórnia; doença pulmonar; escore clínico



## **COMPARISON BETWEEN THE CALIFORNIA CLINICAL ASSESSMENT SCORE AND THORACIC RADIOGRAPHY IN THE DIAGNOSIS OF RESPIRATORY DISEASE IN CALVES**

The global production of cattle is concentrated in a few specific countries. Considered the country with the fastest growing rate of cattle herd, Brazil is the second-largest producer and with the largest growth potential - with more than 53 million calves expected in 2022. Calves get sick primarily due to management problems, which can lead to diarrhea, omphalitis and respiratory diseases often underdiagnosed within the farms. The goal of this study was to analyze the diagnostic technique of clinical evaluation for respiratory disease using the California Clinical Assessment Score, verifying its effectiveness through comparison with thoracic radiography findings. We evaluated 17 calves between 1 and 150 days of life, seemingly suffering from respiratory disease and admitted between August 2021 and September 2022 at the Livestock Development Center of the Federal University of Bahia, located in Santo Amaro, state of Bahia - Brazil. The animals were examined and measurements of rectal temperature, cough, nasal and eye discharge, head and ear position, and abnormal breathing pattern were each scored from 0 to 5 according to the California Clinical Assessment Score described by Love et al. 2014. The right and left lateral decubitus thoracic radiographs were used as a frame of reference for this study, with values adjusted to 2.5 mAs and 100 Kv. Results showed that 13/17 (76.4%) of the calves were considered positive for respiratory disease according to the California Clinical Score. Furthermore, thoracic radiography showed that 100% of the animals showed alterations in the radiographic patterns and therefore were considered positive



for respiratory disease. The California Clinical Score showed good efficiency in directly detecting sick animals with scores for the presence or absence of clinical symptoms. The animals that did not score sufficiently to be considered positive can be evaluated by the interstitial lung disease radiographics pattern, which shows mild signs of pulmonary damage. Through this study and endorsed by radiographic examination, we can conclude that the California Clinical Score can be considered a suitable alternative diagnostic method as a preliminary assessment in veterinary hospitals and farms, helping early diagnosis and decision making regarding treatment and preventive management.

**Keywords:** Cattle; California; lung disease; clinical score



## **COMPARAÇÃO ENTRE O ESCORE DE AVALIAÇÃO CLÍNICA DE WISCONSIN E A RADIOGRAFIA TORÁCICA NO DIAGNÓSTICO DA DOENÇA RESPIRATÓRIA DE BEZERROS**

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O Brasil atua no cenário mundial como o país que mais cresce seu rebanho de bovinos. Em 2022 estima-se crescimento de 53,3 milhões de cabeças de bezerros. As doenças respiratórias representam parcela expressiva das enfermidades desta classe, como afecções primárias ou secundárias às onfalites e diarreias, embora o diagnóstico da doença respiratória dos bezerros permaneça negligenciado. Objetivou-se com esse estudo analisar a técnica diagnóstica do escore de avaliação clínica de Wisconsin para doença respiratória verificando sua capacidade de identificação através da comparação com os achados na radiografia torácica. Foram avaliados 17 bezerros entre 1 e 150 dias de vida, com queixa de doença respiratória, admitidos entre agosto de 2021 e setembro de 2022 no Centro de Desenvolvimento da Pecuária da Universidade Federal da Bahia, localizado em Santo Amaro, estado da Bahia. Os pacientes foram examinados e os sinais clínicos temperatura retal, tosse, secreção nasal, ocular e o seu tipo e a posição de cabeça e orelhas foram graduados de 0 a 3 pontos para cada sinal de acordo com o escore clínico de Wisconsin descrito por



McGuirk, 2008. A radiografia torácica em decúbito latero-lateral direito e esquerdo foi o exame referência para este estudo, com os valores ajustados em 2,5 mAs e 100 Kv. A partir dos resultados constatou-se que 5/17 (29,4%) dos animais avaliados pontuaram indicando doença respiratória de acordo com escore clínico de Wisconsin. E 100% dos bezerros foram positivos para doença respiratória, de acordo com a radiografia torácica que indicou padrões de imagem radiográfica alterados. A graduação das pontuações do escore clínico de Wisconsin pode ser um fator que influenciou na baixa pontuação dos animais que não atingiram os 5 pontos que indicam positivo. A ausência do parâmetro ausculta pulmonar alterada pode ser outro limitante, visto que 14/17 (82,3%) dos bezerros apresentaram este sinal. A partir das análises, embora o escore clínico de Wisconsin seja bastante utilizado sugerem-se melhorias no método de forma a elevar sua sensibilidade na avaliação clínica dos bezerros.

**Palavras-chave:** Bovinos; doença pulmonar; escore clínico; Wisconsin



## **COMPARISON BETWEEN THE WISCONSIN CLINICAL ASSESSMENT SCORE AND THORACIC RADIOGRAPHY IN THE DIAGNOSIS OF RESPIRATORY DISEASE IN CALVES**

Considered the country with the fastest growing rate of cattle herd, Brazil is the second-largest producer and with the largest growth potential - with more than 53 million calves expected in 2022. Respiratory diseases are a big portion of the diseases of this rank, occurring mostly as primary or secondary disorders to omphalitis and diarrhea, although calf respiratory disease diagnosis is still neglected. This study aimed to analyze the diagnostic technique of clinical evaluation for respiratory disease using the Wisconsin Clinical Assessment Score, verifying its effectiveness through comparison with thoracic radiography findings. We evaluated 17 calves between 1 and 150 days of life, seemingly suffering from respiratory disease and admitted between August 2021 and September 2022 at the Livestock Development Center of the Federal University of Bahia, located in Santo Amaro, state of Bahia - Brazil. The animals were examined and measurements of rectal temperature, cough, nasal and eye discharge and their types, as well as head and ear position were each scored from 0 to 3 according to the Wisconsin clinical score described by McGuirk, 2008. The right and left lateral decubitus thoracic radiographs were used as a frame of reference for this study, with values adjusted to 2.5 mAs and 100 Kv. Results showed that 5/17 (29.4%) of the calves were found to be positive for respiratory disease according to the Wisconsin Clinical score. However, thoracic radiography showed that 100% of the animals evaluated showed alterations in the radiographic patterns and therefore were





considered positive for respiratory disease. The grading method of the Wisconsin evaluation may be a factor that influenced the low score of the animals that haven't attained 5 points, which would indicate calf respiratory disease. The absence of the altered pulmonary auscultation may be another limiting factor since 14/17 (82.3%) of the calves presented this symptom. The results indicate that although the Wisconsin clinical score is widely used, methodological improvements are needed in order to increase the sensitivity of the clinical evaluation of calves.

**Keywords:** Cattle; lung disease; clinical score; Wisconsin



## **DISTRIBUIÇÃO DAS CONSOLIDAÇÕES PULMONARES EM BEZERRAS COM DOENÇA RESPIRATÓRIA BOVINA**

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A ultrassonografia torácica é uma técnica que permite o diagnóstico de casos clínicos e subclínicos de doença respiratória bovina (DRB) ao possibilitar a visualização de áreas de irregularidades pleurais e consolidações pulmonares no animal vivo. O objetivo do trabalho foi de avaliar a distribuição das consolidações pulmonares em cada lobo pulmonar de bezerras da raça holandês pela técnica de ultrassonografia torácica. Em uma fazenda de produção leiteira comercial do centro-oeste brasileiro, sob o protocolo CEUA UFMG nº 358/2019, foram avaliadas 193 bezerras da 1<sup>a</sup> a 24<sup>a</sup> semana de vida, totalizando 1158 avaliações em diferentes momentos. Em 466 avaliações dos lobos pulmonares positivas na ultrassonografia, 25% (117/466) apresentaram consolidações em ambos os pulmões, 72,7% (339/466) apenas no pulmão direito e 1,7% (8/466) apenas no pulmão esquerdo. A principal localização das consolidações pulmonares foi observada na porção cranial do lobo cranial direito, com 87,6% (408/466) das consolidações encontradas e 42,9% (200/466) destas restritas a



esta região. A porção caudal do lobo cranial direito foi a segunda área mais afetada, com 38,9% (182/466) das lesões, seguido pelo lobo médio (22,4%; 105/466), porção caudal do lobo cranial esquerdo (18,9%; 88/466), porção cranial do lobo cranial esquerdo (15,8%; 74/466) e por fim, os lobos caudais esquerdo (4,9%; 23/466) e direito (4,5%; 21/466). Os lobos caudais foram os menos afetados, apresentando lesões exclusivas em apenas 1,0% (5/466) das avaliações, enquanto nos demais casos as lesões iniciaram nos lobos craniais e se estenderam até as porções caudais. O lobo cranial normalmente é o mais acometido em bezerros com broncopneumonia, pois é o primeiro a receber os patógenos das vias aéreas superiores, além de ser aerado através do brônquio traqueal, uma pequena abertura na traqueia localizada cranialmente à bifurcação da carina, o que dificulta a depuração mucociliar pela localização e tamanho. Quando a ultrassonografia torácica é utilizada a nível de rebanho para monitorar a DRB, é possível restringir a análise aos lobos craniais para conferir mais agilidade ao exame e aumentar a sensibilidade da técnica.

**Palavras-chave:** Lobo cranial; Monitoramento; Ultrassonografia pulmonar



## DISTRIBUTION OF PULMONARY CONSOLIDATIONS IN CALVES WITH BOVINE RESPIRATORY DISEASE

Thoracic ultrasound is a technique that allows the diagnosis of clinical and subclinical cases of bovine respiratory disease (BRD) by enabling the visualization of areas of pleural irregularities and pulmonary consolidations in the live animal. The aim of this study was to evaluate the distribution of pulmonary consolidations in each lung lobe of Holstein calves using thoracic ultrasound. In a commercial dairy farm in the Brazilian Midwest, under the protocol CEUA UFMG nº 358/2019, 193 calves from the 1st to 24th week of life were evaluated, totaling 1158 evaluations at different times. In 466 ultrasound-positive lung lobe assessments, 25% (117/466) had consolidation in both lungs, 72.7% (339/466) in the right lung only, and 1.7% (8/466) in the left lung only. The main location of pulmonary consolidations was observed in the cranial portion of the right cranial lobe, with 87.6% (408/466) of the consolidations found and 42.9% (200/466) of them restricted to this region. The caudal portion of the right cranial lobe was the second most affected area, with 38.9% (182/466) of lesions, followed by the middle lobe (22.4%; 105/466), caudal portion of the left cranial lobe (18.9%; 88/466), cranial portion of the left cranial lobe (15.8%; 74/466) and finally, the left caudal lobes (4.9%; 23/466) and right (4.5%; 21/466). The caudal lobes were the least affected, presenting exclusive lesions in only 1.0% (5/466) of the evaluations, while in the other cases the lesions started in the cranial lobes and



extended to the caudal portions. The cranial lobe is usually the most affected in calves with bronchopneumonia, as it is the first to receive pathogens from the upper airways, in addition to being aerated through the tracheal bronchus, a small opening in the trachea located cranially to the bifurcation of the carina, which makes it difficult to mucociliary clearance by location and size. When thoracic ultrasound is used at the herd level to monitor BRD, it is possible to restrict the analysis to the cranial lobes to make the examination more agile and increase the sensitivity of the technique.

**Key words:** Cranial lobe; Monitoring; Thoracic ultrasound



## DOENÇA RESPIRATÓRIA EM BOVINOS DE LEITE ASSOCIADA A INFECÇÕES POR GAMMA HERPESVÍRUS OVINO 2, GAMMA HERPESVIRUS BOVINO 6 E CORONAVÍRUS BOVINO

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O gamma herpesvirus ovino (OvGHV2) é membro do gênero *Macavirus*, faz parte do grupo dos vírus da febre catarral maligna (VFCM) que produz a febre catarral maligna (FCM) em diversos hospedeiros mamíferos. Entretanto, a função do OvGHV2 como causa da doença respiratória bovina (BRD) é incipiente e não está bem estabelecida. Este estudo investigou a causa de um surto de doença respiratória seguida por diarreia em um rebanho de bovino leiteiro no sul do Brasil. Swabs nasais profundos (SNP) foram coletados de bezerros assintomáticos (n=2), bezerros com desconforto pulmonar (n=6) e bezerros diarreicos após episódios de desconforto respiratório (n=7) para a detecção molecular dos principais patógenos associados a BRD. Além disso, 11 amostras fecais foram coletadas para à detecção molecular de doenças entéricas de bovinos. Adicionalmente, tecidos pulmonares de bezerros (n=3) e de uma vaca



que morreu foram recebidos para avaliação molecular; fragmentos intestinais e pulmonares de um bezerro e da vaca foram analisados histologicamente. A histopatologia revelou pneumonia intersticial e enterite atrófica nos dois animais. A imuno-histoquímica (IHQ) identificou antígenos intralesionais de um VFCM no citoplasma das células epiteliais dos pulmões e intestinos. PCR amplificou o DNA do OvGHV2 da maioria (93,3%; 14/15) do SNP e de todos os fragmentos pulmonares e intestinais dos bezerros e da vaca que morreu, confirmando que o *Macavírus* identificado por IHQ era OvGHV2. Além disso, houve infecções pulmonares concomitantes de OvGHV2 com gamaherpesvírus bovino 6 (n=2) e coronavírus bovino (BCoV; n=1). Adicionalmente, *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*, vírus da diarreia viral bovina (BVDV), vírus respiratório sincicial bovino, alfaherpesvírus bovino 1 e vírus da parainfluenza bovina 3 não foram identificados no SNP e nos pulmões. Análises de RT-PCR amplificaram BVDV1b (n=4) e Aichivirus B (n=2) nas amostras fecais que não continham rotavírus A e BCoV. Esses achados demonstraram que o OvGHV2, um Macavírus, foi o agente infeccioso frequentemente detectado em bovinos de leite com manifestação clínica e histológica de pneumonia e foi associado à síndrome respiratória aqui descrita. Consequentemente, sugere-se que o OvGHV2 seja considerado como causa de DRB e deve ser incluído no diagnóstico diferencial de bovinos com síndrome respiratório.

**Palavras chaves:** doença pulmonar; diagnóstico imuno-histoquímico; *Macavírus*; OvGHV2



## **BOVINE RESPIRATORY DISEASE IN DAIRY CATTLE ASSOCIATED WITH INFECTIONS BY OVINE GAMMAHERPESVIRUS 2, BOVINE GAMMAHERPESVIRUS 6, AND BOVINE CORONAVÍRUS**

Ovine gammaherpesvirus (OvGHV2) is member of the *Macavirus* genus, is part of the malignant catarrhal fever virus (MCFV) group that produces malignant catarrhal fever (MCF) in several mammalian hosts. However, the role of OvGHV2 as a cause of bovine respiratory disease (BRD) is incipient and not well established. This study investigated the cause of an outbreak of respiratory disease syndrome followed by diarrhea in a dairy cattle herd from Southern Brazil. Deep nasal swabs (DNS) were collected from asymptomatic calves ( $n=2$ ), calves with pulmonary discomfort ( $n=6$ ), and diarrheic calves after episodes of respiratory distress ( $n=7$ ) for the molecular detection of the principal pathogens associated with BRD. Furthermore, 11 fecal samples were collected for the molecular detections of enteric diseases of cattle. Additionally, pulmonary tissues from calves ( $n=3$ ) and a cow that died were received for molecular evaluation; fragments of the intestine and lungs of one calf and the cow were analysed histologically. Histopathology revealed interstitial pneumonia and atrophic enteritis in both animals. An immunohistochemical (IHC) assay identified intralésional antigens of a MCFV within epithelial cells of the lungs and intestines. PCR assays amplified OvGHV2 DNA from most (93.3%; 14/15) of the DNS, and all pulmonary and intestinal fragments from the calves and the cow that died,





confirming that the *Macavirus* identified by IHC was OvGHV2. Additionally, there were concomitant pulmonary infections of OvGHV2 with bovine gammaherpesvirus 6 ( $n=2$ ) and bovine coronavirus (BCoV;  $n=1$ ). Furthermore, *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*, bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus, bovine alphaherpesvirus 1, and bovine parainfluenza virus 3 were not identified from the DNS and lungs. RT-PCR assays amplified BVDV1b ( $n=4$ ) and Aichivirus B ( $n=2$ ) in the fecal samples that did not contain rotavirus A and BCoV. These findings demonstrated that OvGHV2, a *Macavirus*, was the infectious disease agent frequently detected in dairy cattle with clinical and histological evidence of pneumonia and was associated with the respiratory syndrome herein described. Consequently, it is suggested that OvGHV2 should be considered as a cause of BRD and be included in the differential diagnosis of cattle with respiratory distress syndromes.

**Keywords:** pulmonary disease; diagnostic immunohistochemistry; *Macavirus*; OvGHV2



## **EFEITO DO *ENTEROCOCCUS FAECIUM* E *SACHAROMYCES CEREVISIAE* NA RESPOSTA IMUNE BRONCOALVEOLAR DE BEZERRAS VACINADAS CONTRA O COMPLEXO RESPIRATÓRIO BOVINO**

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Embora vacinas intranasais contra complexo respiratório bovino (CRB) contendo vírus atenuado provoquem maior estimulação de resposta humoral local, elas reduzem a defesa celular durante o estabelecimento da imunidade vacinal. Probióticos contendo *S cerevisiae* ou *E faecium* reduziram a ocorrência de CRB em bezerras neonatas desafiados com vírus respiratório sincicial bovino e potencializaram a resposta humoral após a vacinação em modelos murino, gerando-se a dúvida se poderiam ter o mesmo efeito em bezerros. Este trabalho teve o objetivo de verificar se o probiótico contendo *E faecium* e *S cerevisiae* atenua a inflamação causada pela vacina na região do trato respiratório em 24 bezerras da raça Jersey, sadias entre 6 a 7 meses de idade. Para tanto, as bezerras foram divididas nos grupos: controle (C), suplementado (S), vacinado (V) e suplementado e vacinado (SV), com 6 animais em cada. A suplementação ocorreu durante 56 dias (Probios precise®, Ouro Fino®, 2g/dia/animal). A vacinação intranasal foi realizada em dose única no dia 15 (Inforce®, Zoetis®,



1ml/ narina). Nos períodos D0, D18, D22 e D36 realizou-se avaliações endoscópicas do trato respiratório; e análises citológica broncoalveolares (BA) e mensuração de IgA BA. Os grupos vacinados apresentaram maior irritação da nasofaringe e traqueia em D18 e D22. O grupo V apresentou redução da produção de ERO por fagócitos BA e aumento da celularidade por influxo neutrofílico na região em D18 e D22. O SV apresentou aumento de produção ERO por fagócitos BA nos dias 18 e 22, com manutenção da celularidade local. Em D36, os grupos vacinados apresentaram aumento de celularidade local por influxo de neutrófilo. Em D22 houve aumento da produção de IgA BA, para os grupos S, V e SV. Em D36 houve aumento de IGA BA nos grupos vacinados, sendo o SV maior que o aumento do V. Conclui-se que a suplementação com *E faecium* e *S cerevisiae* isolada promoveu aumento de produção de IGA BA e em associação com a vacina, atenuou a inflamação do trato respiratório produzida pela mesma, evitando a redução da imunidade celular local, além de potencializar a resposta humoral da vacina contendo vírus atenuado contra o CRB.

**Palavras-chave:** macrófago alveolar, espécies reativas de oxigênio, IgA, broncoscopia



## **EFFECT OF *ENTEROCOCCUS FAECIUM* AND *SACCHAROMYCES CEREVISIAE* ON THE BRONCHOALVEOLAR IMMUNE RESPONSE OF CALVES VACCINATED AGAINST THE BOVINE RESPIRATORY COMPLEX**

Although intranasal bovine respiratory complex (BRC) vaccines containing attenuated virus promote a higher local humoral response, they reduce cellular defense during the establishment of vaccine immunity. Probiotics containing *S cerevisiae* or *E faecium* reduced the occurrence of BRC in neonate calves challenged with bovine respiratory syncytial virus and potentiated the humoral response after vaccination in murine models. It is possible they could have the same effect in calves. This study aimed to verify whether the probiotic containing *E faecium* and *S cerevisiae* attenuates the inflammation caused by the vaccine in the respiratory tract in 24 healthy Jersey calves (6±1 mo). The calves were divided into groups with 6 animals in each: control (C), supplemented (S), vaccinated (V) and supplemented and vaccinated (SV). Supplementation occurred during 56 days (Probios precise®, Ouro Fino®, 2g/day/animal). Intranasal vaccination was performed as a single dose on day 15 (Inforce®, Zoetis®, 1ml/nostril). On days 0, 18, 22 and 36, endoscopic evaluations of the respiratory tract, bronchoalveolar (BA) cytological analysis and measurement of BA IgA. were performed. The vaccinated groups showed higher irritation of the nasopharynx and trachea on days 18 and 22. V Group showed reduction of ROS



production by BA phagocytes and an increase of cellularity by neutrophilic influx in the region on days 18 and 22. SV showed an increase of the ROS production by BA phagocytes on days 18 and 22, and maintenance of the local cellularity. On day 36, the vaccinated groups showed an increase of the cellularity due to neutrophil influx in BA. On day 22 there was an increase of the IgA BA in the S, V and SV groups. On day 36, there was an increase of the BA IgA of the vaccinated groups, BA IgA of SV was higher than V group. It is concluded the *E. faecium* and *S. cerevisiae* supplementation alone promoted an increase of the BA IgA production and, when it was associated with the vaccine, it attenuated the inflammation of the respiratory tract produced by vaccine, it prevented the reduction of the local cellular immunity, and it potentiated the humoral response caused by the vaccine containing attenuated virus against BRC.

**Keywords:** alveolar macrophage, reactive oxygen species, IgA, bronchoscopy



## **ESTUDO RETROSPECTIVO DOS CASOS DE TUBERCULOSE BOVINA DIAGNOSTICADOS NA FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA DA UNIVERSIDADE DE SÃO PAULO ENTRE 2002 E 2022**

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A tuberculose bovina é uma zoonose de ocorrência mundial, que representa grandes riscos à saúde da população que consome produtos de origem animal e determina grandes prejuízos à pecuária. Atualmente a doença encontra-se em controle rigoroso em muitos países desenvolvidos, mas ainda é a maior causa de perda em rebanhos em muitos países subdesenvolvidos. O objetivo deste resumo foi de realizar estudo retrospectivo dos casos de tuberculose bovina diagnosticados entre 2002 a 2022 na Clínica de Bovinos e Pequenos Ruminantes da Faculdade de Medicina Veterinária da Universidade de São Paulo. A distribuição dos casos positivos revelou que 100% dos 41 casos foram provenientes do Estado de São Paulo, sendo distribuídos entre os municípios de São Paulo (48,78%, 20/41), Atibaia, (12,19%, 5/41), Campinas (2,43%, 1/41), Pirassununga (2,43%, 1/41), Vargem Grande Paulista (2,43%, 1/41), destacando-se os municípios do sudoeste da região metropolitana de São Paulo (12,19%, 5/41). Não foi encontrada a origem em 19,51% (8/41) dos casos. Em



65,85% (27/41), a idade era acima de dois anos, 17,07% (7/41) entre um e dois anos, 12,19% (5/41) inferior a um ano, 2,43% (1/41) acima de 20 anos e 2,43% (1/41) não se sabia a idade. As queixas mais comumente relatadas na anamnese foram: emagrecimento progressivo (14,63%), tosse (9,75%), dispneia (7,31%) e secreção nasal (4,87%). O teste cervical comparativo foi realizado em 26 animais, revelando que 46,15% apresentaram reação considerada inconclusiva ao teste e 53,84% apresentaram reação considerada positiva. A não realização dos testes de tuberculina em 40% (16/41) dos bovinos estava associada ao fato de que estes animais não apresentaram manifestações clínicas sugestivas da tuberculose bovina. Dentre os animais eutanasiados e/ou que foram a óbito, os achados anatomopatológicos mais comumente observados foram: 100% apresentavam lesões granulomatosas em diversos órgãos, com abscessos de conteúdo purulento e caseificado, e lesões disseminadas de caráter miliar sobre a superfície pleural e peritoneal. A tuberculose bovina é uma importante zoonose no contexto da saúde única, além de também causar significativo impacto na bovinocultura, fazendo-se necessário reforçar as medidas de controle, prevenção e diagnóstico.

**Palavras-chave:** doença respiratória; ruminantes; zoonoses



## **RETROSPECTIVE STUDY OF BOVINE TUBERCULOSIS CASES DIAGNOSED AT THE SCHOOL OF VETERINARY MEDICINE AND ANIMAL SCIENCE, UNIVERSITY OF SÃO PAULO FROM 2002 TO 2022**

Bovine tuberculosis is an worldwide zoonosis, which represents great risks to the health of the population that consumes products of animal origin and determines great damages to livestock. Currently, the disease is under strict control in many developed countries, but it is still the biggest cause of loss in herds in many underdeveloped countries. The objective of this abstract was to carry out a retrospective study of bovine tuberculosis cases diagnosed from 2002 to 2022 at the Cattle and Small Ruminants Clinic of the School of Veterinary Medicine, University of São Paulo. The positive cases revealed that 100% of the 41 cases were from the State of São Paulo, mainly from the cities of São Paulo (48.78%, 20/41), Atibaia, (12.19%, 5/41), Campinas (2.43%, 1/41), Pirassununga (2.43%, 1/41), Vargem Grande Paulista (2.43%, 1/41), with emphasis on the municipalities in the southwest of the metropolitan region of São Paulo (12.19%, 5/41). The origin was not found in 19.51% (8/41) of the cases. In 65.85% (27/41), the age was older than two years old, 17.07% (7/41) between one and two years old, 12.19% (5/41) less than one year old, 2.43% (1/41) were over 20 years old and in 2.43% (1/41), the age was not known. The most commonly reported complaints in the anamnesis were: progressive weight loss (14.63%), cough (9.75%), dyspnea (7.31%) and nasal discharge (4.87%). The comparative cervical test was performed in 26 animals: 46.15% presented an inconclusive





reaction and 53.84% presented a positive reaction. Failure to perform tuberculin tests in 40% (16/41) of cattle was due to the lack of clinical manifestations suggestive of bovine tuberculosis. Among the animals euthanized and/or that died, the pathological findings most commonly observed were: 100% had granulomatous lesions in several organs, with abscesses with purulent and caseous content, and disseminated miliary lesions on the pleural and peritoneal surface. Bovine tuberculosis is an important zoonosis in the context of one health, has a significant impact on beef and dairy cattle production, and it is necessary to strengthen control, prevention and diagnosis measures.

**Keywords:** respiratory disease; ruminants; zoonosis



## FENOTIPAGEM DAS CÉLULAS MONONUCLEARES DO LAVADO BRONCO ALVEOLAR (LBA) EM BEZERROS ESTIMULADOS COM *MANNHEIMIA HAEMOLYTICA*.

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A doença respiratória bovina representa um gargalo na produção de bovinos, principalmente nas fases de cria e recria. A *Mannheimia haemolytica*, é a principal bactéria isolada de bovinos com doença respiratória. O trato respiratório bovino possui como principal célula de defesa o macrófago alveolar residente, responsável pela homeostase imunológica do pulmão. O objetivo deste estudo foi identificar e avaliar a população de leucócitos alveolares de bezerros por meio de modelo de infecção experimental com *Mannheimia haemolytica*. Para tal, foram utilizados 12 bezerros machos, hígidos, que foram infectados com *M. haemolytica*, com dose de 5 mL contendo  $1 \times 10^9$  unidades formadoras de colônias (UFC), infundida na luz da traqueia. Os animais foram distribuídos aleatoriamente, em dois grupos, seis cada: Grupo 1 (G1): tratamento com norfloxacin e flunixin meglumina; Grupo 2 (G2): tratamento com norfloxacin. O lavado bronco alveolar (LBA) foi realizado por endoscopia em quatro momentos: Momento 1 (M1): dois dias pré-infecção; Momento 2 (M2): pós-infecção e aparecimento dos sintomas; Momento 3 (M3): um dia pós-tratamento;



Momento 4 (M4): sete dias pós-tratamento. A quantificação e identificação das populações celulares foi realizada por citometria de fluxo. A análise estatística foi realizada utilizando o *software* GraphPad Prism®, que considerou significativas as análises que apresentaram  $P \leq 0,05$ . Nos resultados, observou-se que os leucócitos CD4/CD8+ não vacuolizados reduziram no G2 no M2 em relação ao M1 e M4 ( $P=0,0321$ ). Enquanto, CD4/CD8+ vacuolizados diminuiu em M2 em relação à M4, em G1 ( $P=0,0049$ ) e G2 ( $P=0,0085$ ). O uso ou não de anti-inflamatório não interferiu nas populações avaliadas. A análise dos mononucleares totais do LBA apresentaram diferença no G2, onde o M1 apresentou redução de células em relação ao M4 ( $P=0,043$ ). Observando a população de CD14+ notou-se uma menor porcentagem de células no M2 em relação ao M4 dos dois grupos experimentais ( $P=0,0009$  e  $0,0007$  G1 e G2 respectivamente). Na quantificação das populações expressando TLR4 o G1 apresentou diferença entre os momentos, onde as populações em M2 foram menores em relação as populações em M4 ( $P=0,029$ ). Conclui-se que as alterações funcionais dos fagócitos apresentam papel importante na patogenia da manheimiose, com destaque para a subpopulações de CD8+.

**Palavras-chave:** Doença respiratória bovina, Fagócitos alveolares, infecção experimental, Resposta imune



## PHENOTYPING OF BRONCHO ALVEOLAR LAVAGE (BAL) MONONUCLEAR CELLS IN CALVES STIMULATED WITH *MANNHEIMIA HAEMOLYTICA*.

Bovine respiratory disease represents a challenge in cattle production, especially in the breeding and rearing phases. *Mannheimia haemolytica* is the main bacterium isolated from cattle with respiratory disease. Bovine respiratory tract has as its main defense cell the resident alveolar macrophage, responsible for the immunological homeostasis of the lung. The objective of this study was to identify and evaluate the population of alveolar leukocytes of calves through an experimental infection model with *Mannheimia haemolytica*. For this, 12 healthy male calves were used, which were infected with *M. haemolytica*, with a dose of 5 mL containing  $1 \times 10^9$  colony forming units (CFU), infused in the tracheal lumen. Animals were randomly divided into two groups, six each: Group 1 (G1): treatment with norfloxacin and flunixin meglumine; Group 2 (G2): treatment with norfloxacin. Bronchoalveolar lavage (BAL) was performed by endoscopy at four moments: Moment 1 (M1): two days pre-infection; Moment 2 (M2): post-infection and onset of symptoms; Moment 3 (M3): one day post-treatment; Moment 4 (M4): seven days post-treatment. The quantification and identification of cell populations was performed by flow cytometry. Statistical analysis was performed using the GraphPad Prism® software, which considered significant the analyzes that presented  $P < 0.05$ . In the results, it was observed that non-vacuolized



CD4/CD8+ leukocytes reduced in G2 in M2 in relation to M1 and M4 ( $P=0.0321$ ). While, vacuolated CD4/CD8+ decreased in M2 compared to M4, in G1 ( $P=0.0049$ ) and G2 ( $P=0.0085$ ). The use or not of anti-inflammatory drugs did not affect the populations evaluated. The analysis of total mononuclear cells from BAL showed differences in G2, where M1 showed a reduction of cells in relation to M4 ( $P=0.043$ ). Observing the CD14+ population, a lower percentage of cells was observed in M2 in relation to M4 in the two experimental groups ( $P=0.0009$  and  $0.0007$  G1 and G2 respectively). In quantification of the populations expressing TLR4, the G1 showed difference between the moments, where the populations in M2 were smaller in relation to the populations in M4 ( $P=0.029$ ). It is concluded that the functional alterations of phagocytes play an important role in the pathogenesis of mannheimiosis, with emphasis on CD8+ subpopulations.

**Keywords:** Bovine respiratory disease, Alveolar phagocytes, Experimental infection, Immune response



## ISOLAMENTO DO VÍRUS SINCICIAL RESPIRATÓRIO BOVINO (BRSV) E ASSOCIAÇÃO COM *Pasteurella multocida* EM QUADRO DE DOENÇA RESPIRATÓRIA BOVINA (BRD).

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O complexo de doenças respiratórias bovinas (BRDC) é um dos principais problemas enfrentados pelos produtores com perdas econômicas relacionadas a tratamento e controle, em muitos casos sem sucesso, ocasionando o óbito do animal. Dentre os principais patógenos virais destacam-se o vírus da diarreia viral bovina (BVDV), alpha-Herpesvirus bovino -1, vírus sincicial respiratório bovino (BRSV) e vírus da parainfluenza bovina tipo 3 (bPI3). Entretanto, ao longo dos anos, novos vírus têm sido identificados como o vírus influenza D, coronavírus bovino, rinovírus tipos A e B. O BRDC também pode apresentar associação com agentes bacterianos como *Pasteurella multocida*, *Mannheimia haemolytica* e *Histophilus somni*. O presente trabalho descreveu problemas respiratórios em criação de bovinos na região Sudeste do Estado de São Paulo. Cinco bovinos com sintomas respiratórios como: febre, coriza serosa com evolução mucopurulenta e tosse produtiva. Amostras de suabe nasal em solução fisiológica a 0,9%, foram encaminhadas aos laboratórios de Bacteriologia Geral e Viroses de Bovídeos do Instituto Biológico, para diagnóstico bacteriológico e viral de doenças respiratórias. Foi realizada a PCR para detecção da *P*



*multocida*. com alvo no gene KMT1 e amplificação de fragmento de 460 pb. O diagnóstico molecular para BRSV e PI3 por qPCR utilizou set de primers e probes dos genes N e M, respectivamente. As amostras positivas na RT-qPCR foram submetidas ao isolamento viral em monocamada de células Madin Darby Bovine Kidney (MDBK), mantidas em estufa a 37°C com 5% de CO<sub>2</sub> por 5 dias. Das cinco amostras de suabe, 2/5(40%) foram positivas para *P. multocida*, 5/5 (100%) amostras foram positivas na RT-qPCR para BRSV e 1/5 (20%) amostra foi confirmada no isolamento viral para BRSV com aparecimento de células refringentes e sinciciais, e nenhuma amostra foi positiva para PI3 no teste de RT-qPCR. Este é o primeiro relato de isolamento de vírus sincicial respiratório bovino e detecção de *P. multocida* em amostras de suabes nasais de bovinos no Estado de São Paulo. O diagnóstico diferencial possibilitou a detecção de associação de vírus e bactérias em um surto de doença respiratória bovina, contribuindo para o conhecimento epidemiológico e para a tomada de medidas de tratamento e controle dos agentes envolvidos.

**Palavras chaves:** pneumonia, co-infecção, diagnóstico



## **BOVINE RESPIRATORY DISEASE (BRD) REPORT: BOVINE RESPIRATORY SYNCYTIAL VIRUS (BRSV) ASSOCIATED WITH *Pasteurella multocida***

Bovine respiratory disease complex (BRDC) is one of the main problems faced by the farmer with economic losses related to treatment and control, often with no success, leading to animal death. Among the main viral pathogens there are bovine viral diarrhea virus (BVDV), bovine alpha-Herpesvirus 1 (BoVH-1), bovine respiratory syncytial virus (BRSV) and bovine parainfluenza 3 (bPI3). However, along the years, other virus were identified as Influenza D, bovine Coronavirus, Rhinovirus types A and B. BRDC can also present association with bacterial pathogens as *Pasteurella multocida*, *Mannheimia haemolytica* and *Histophilus somni*. The present report described respiratory syndroms in a bovine production system from São Paulo State, southeastern Brazil. Five animals with respiratory symptoms as fever, serous coryza with mucopurulent evolution and productive cough. Samples of nasal swab suspended in saline solution were processed in the laboratories from Instituto Biológico for bacteriological and viral diagnosis of respiratory diseases. *P. multocida* detection was performed by means of PCR reaction targeting KMT1 gene and 460 pb product amplification. RT-qPCR of BRSV and PI3 used a set of primers and probes for N and M genes, respectively. Viral isolation of the positive samples in the RT-qPCR was performed in Madin Darby Bovine Kidney (MDBK) monolayer cells mantained at 37°C with 5% CO<sub>2</sub> for 5 days. Out of the five swab samples, 2/5 (40%) were positive for *P. multocida*, 5/5 (100%) positive for BRSV by RT-qPCR and 1/5 (20%) confirmed BRSV in





viral isolation that presented refractory and syncytial cells, furthermore no sample was positive for PI3. To our knowledge, this is the first report of BRSV and *P. multocida* association in bovine nasal swabs in São Paulo State. Differential diagnosis allowed bacterial and virus co-infection detection in a bovine respiratory disease outbreak, contributing to epidemiological science and for the correct treatment and control measures.

**Keywords:** pneumonia, co-infection, diagnosis



## MEDIDAS DE BIOSSEGURIDADE ASSOCIADA AO COMPLEXO DA DOENÇA RESPIRATÓRIA BOVINA

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A biosseguridade tem se expressado como um ramo em ascensão quando observado no cenário da produção leiteira, devido à grande gama de pontos de ação para incremento da saúde animal, bem como à ampla capacidade de impacto passível de ser gerado. Nesse sentido, considerando-se os diferentes agentes infecciosos relacionadas ao trato respiratório em bezerras na fase de aleitamento, destacam-se o Herpesvírus Bovino Tipo 1 (BoHV-1) e Vírus da Diarreia Viral Bovina (BVDV), que determinam a imunossupressão e susceptibilidade aos agentes infecciosos bacterianos, potencializando a importância de medidas de biosseguridade, nesse caso, atuantes em cenário de dualidade, haja vista a possibilidade de contenção da infecção viral bem como da doença respiratória. Assim, o presente estudo objetivou mensurar fatores relacionados às doenças respiratórias em rebanhos pertencentes à Frísia Cooperativa Agroindustrial. Para tal, estruturou-se um questionário dividido em duas seções, para captação de informações sobre práticas gerais e específicas



relacionadas ao BoHV-1 e BVDV. Nesse formulário, obteve-se 68 respostas, as quais demonstraram a percepção de não proteção das propriedades em relação aos vírus de enfoque (50,7%; 34/67), assim como a preocupação em relação ao acometimento por BoHV-1 e BVDV (96,9%; 63/65) e em relação à percepção de risco dos produtores quanto às doenças respiratórias, os bezerros foram identificados como a categoria com maior desafio (78,8%; 52/66) seguido das vacas em lactação (16,7; 11/66). Além disso, houve relato de registro de ocorrência de doença respiratória (91,2%; 62/68), possuindo protocolo de tratamento pré-estabelecido (57,4%; 39/68) com predomínio do uso de enrofloxacino e macrolídeo (22,4%; 8/36 para ambos os princípios ativos). A partir dos dados, notou-se o relato de conhecimento relacionados a enfermidades decorrentes do BoHV-1 e BVDV (89,7%; 61/68 e 91,2%; 62/68, respectivamente), assim como acometimentos respiratórios, no entanto, há espaço para ampliação da visão sobre acometimentos pelos agentes citados, uma vez que, em manejos relacionados à reprodução, forma de transmissão do BVDV e BoHV-1, algumas medidas como correta destinação de fetos abortados (6%; 4/67) não são adotadas, situações as quais impactam na biossegurança respiratória de forma indireta. Dessa forma, tendo em vista a base do conceito de biossegurança em assegurar que as propriedades e seus animais tenham melhor saúde por meio do controle de pontos estratégicos, deve-se considerar como ponto de partida o nivelamento do conhecimento sobre doença respiratória assim como o incentivo ao monitoramento visando o status sanitário igualitário à produção leiteira.

**Palavras-chaves:** Biossegurança, Herpesvírus, Doença Respiratória, BVDV



## BIOSECURITY MEASURES ASSOCIATED WITH BOVINE RESPIRATORY DISEASE COMPLEX

Biosecurity has been expressed as a growing field in the dairy production scenario, due to the wide range of action points to increase animal health, as well as the wide impact capacity that can be generated. In this sense, considering the different infectious agents related to the respiratory tract in lactating calves, Bovine Herpesvirus Type 1 (BoHV-1) and Bovine Viral Diarrhea Virus (BVDV) stand out, which determine immunosuppression and susceptibility to bacterial infectious agents, fact that enhance the importance of biosecurity measures, in this case, performing in a duality scenario, with the possibility of containing the viral infection as well as the respiratory disease. Thus, the present study aimed to measure factors related to respiratory diseases in herds belonging to the Frísia Cooperativa Agroindustrial. For that, a questionnaire divided into two sections was structured to obtain information of general and specific practices related to BoHV-1 and BVDV. In this form, 68 responses were obtained, which demonstrated the perception of non-protection of properties in relation to the focus viruses (50.7%; 34/67), as well as the concern regarding involvement by BoHV-1 and BVDV (96.9%; 63/65) and in relation to the producer's risk perception regarding respiratory diseases, calves were identified as the category with the greatest challenge (78.8%; 52/66) followed by lactating cows (16.7%; 11/66). In addition, there were reports of respiratory disease (91.2%; 62/68), with



a pre-established treatment protocol (57.4%; 39/68) with predominance use of enrofloxacin and macrolide (22.4%; 8/36 for both active ingredients). From the data, it was noted the report of knowledge related to diseases resulting from BoHV-1 and BVDV (89.7%; 61/68 and 91.2%; 62/68, respectively), as well as respiratory disorders. However, there is space to broaden the view on involvement by the focuses agents in managements related to reproduction, the form of transmission of BVDV and BoHV-1, some measures such as the correct destination of aborted fetuses (6%; 4/67) that are not adopted, situations which indirectly impact respiratory biosecurity. Thus, in view of the basis of the biosecurity concept in ensuring that properties and their animals have better health through the control of strategic points, one should consider as a starting point the leveling of knowledge about respiratory disease as well as the incentive to monitoring aiming at the egalitarian sanitary status of milk production.

**Keywords:** Biosecurity, Herpesvirus, Respiratory Disease, BVDV



## **OCORRÊNCIA DE DOENÇAS RESPIRATÓRIAS EM BEZERROS DA RAÇA GIROLANDO CRIADOS NO TRÓPICO ÚMIDO AMAZÔNICO**

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Os bezerros, em seus diversos sistemas de criação, são afetados por doenças respiratórias, sobretudo quando se há elevada concentração de agentes patogênicos nos bezerreiros, sendo o período neonatal o mais desafiador da vida do bezerro. Objetivou-se com esse trabalho avaliar a ocorrência de doenças respiratórias em bezerros girolando criados no trópico úmido amazônico. O estudo foi realizado em uma propriedade localizada no município de Paragominas, de clima tropical chuvoso, estado do Pará, no período de dezembro de 2021 a julho de 2022, no qual foram utilizados 80 bezerros, sendo 28 machos e 52 fêmeas, acompanhados desde o nascimento até os 60 dias de idade. Durante os manejos diários, foi realizada ronda sanitária para verificar o estado de saúde dos bezerros, onde diante disso, ao observar alguma alteração,



o animal era examinado, tratado e acompanhado até sua recuperação. A propriedade possuía banco de colostro e ao nascimento de um bezerro era fornecido colostro descongelado em banho-maria a temperatura média de 35 a 40 °C com grau brix de 25%. Quando necessário o colostro era enriquecido com colostro artificial em pó até atingir o grau brix igual a 25%. Para prevenção de onfalopatias o cordão umbilical era tratado com iodo 10% até a mumificação e queda do coto umbilical externo. O diagnóstico era feito pelo exame físico do bezerro, identificando os sintomas dentre os quais, tosse, febre, presença de secreção mucopurulenta, apatia e perda de apetite. Pode-se inferir que a taxa de ocorrência de doenças respiratórias em bezerros foi de 23,75% (19/80), um valor bem maior que os 10% recomendados para ocorrência de problemas respiratórios pelo padrão ouro para criação de bezerras e novilhas leiteiras na fase de aleitamento. Destaca-se a importância do manejo sanitário preventivo, sobretudo acerca da conscientização e educação sanitária dos tratadores sobre a adoção de boas práticas de higiene, manejo e biossegurança nas propriedades, com viés de prevenção das doenças respiratórias nos bezerros.

**Palavras-chave:** Amazônia, Enfermidade, Manejo, Neonato



## **OCCURRENCE OF RESPIRATORY DISEASES IN GIROLANDO CALVES BRED IN THE HUMID TROPICS AMAZON**

Calves, in their various rearing systems, are affected by respiratory diseases, especially when there is a high concentration of pathogens in heifers, and the neonatal period is the most challenging of the calf's life. The objective of this work was to evaluate the occurrence of respiratory diseases in girolando calves reared in the humid Amazonian tropics. The study was carried out in a property located in the municipality of Paragominas, with a rainy tropical climate, Pará state, from December 2021 to July 2022, in which 80 calves were used, 28 males and 52 females, followed from birth to 60 days of age. During the daily management, a health round was held to verify the health status of the calves, where, in view of this, when observing some alteration, the animal was examined, treated and monitored until its recovery. The property had colostrum bench and at the birth of a calf was provided thawed colostrum in a water bath at an average temperature of 35 to 40 °C with brix degree of 25%. When necessary the colostrum was enriched with artificial colostrum powder until it reached the brix degree equal to 25%. For the prevention of ophnopathies, the umbilical cord was treated with 10% iodo until mummification and fall of the external umbilical stump. The diagnosis was made by physical examination of the calf, identifying the symptoms among which, cough, fever, presence of mucopurulent secretion, apathy and loss of appetite. It can be inferred that the rate of occurrence of respiratory diseases in calves was 23.75% (19/80), a value much higher than the 10% recommended for





the occurrence of respiratory problems by the gold standard for the rearing of heifers and dairy heifers in the breastfeeding phase. The importance of preventive health management is highlighted, especially regarding the awareness and health education of the handlers about the adoption of good hygiene practices, management and biosecurity in the properties, with bias in the prevention of respiratory diseases in calves.

**Keywords:** Amazon, Disease, Management, Neonate



## PLEUROPNEUMONIA INTERSTICIAL AGUDA SEVERA EM VACA GIROLANDO: RELATO DE CASO

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As doenças respiratórias possuem grande capacidade de transmissão, principalmente em sistemas de confinamento, dentre as pneumonias, as mais frequentes são as broncopneumonias e as pneumonia intersticiais, essas por sua vez causam inflamação pulmonar decorrente de infecções virais ou inalação de toxinas. Objetiva-se relatar um caso de pleuropneumonia intersticial em vaca Girolando prenha de cinco anos, proveniente de *Compost Barn* de Jerônimo Monteiro/ES, atendida no Setor de Animais de Produção do Hospital Veterinário da Universidade Federal do Espírito Santo, Campus Alegre/ES. Na anamnese relatou outros casos de pneumonia em diferentes categorias de animais. Clinicamente, observou-se apatia, anorexia, desidratação e enoftalmia. O quadro respiratório grave demonstrou dispneia mista com áreas de abafamento e crepitação na auscultação pulmonar, com aumento do campo na percussão torácica direita, além de aumento de volume dorsal evidenciando enfisema subcutâneo que se estendia da região dorsal cervical até a toracolombar. No



exame ultrassonográfico, verificou-se a presença de grande quantidade de gás, sobretudo em hemitórax direito, associado a presença de moderada quantidade de conteúdo anecogênico entremeado em lobos pulmonares. O hemograma revelou hiperfibrinogenemia, desvio nuclear de neutrófilos à esquerda leve e regenerativo, assim como neutrófilos tóxicos com núcleo em formato de *donuts*. Na bioquímica evidenciou aumento das concentrações séricas de proteína total, glubulina e ureia, além de hipoalbuminemia. Apesar do tratamento, houve declínio do quadro clínico do animal que veio à óbito no dia seguinte. Os achados anatomopatológicos pulmonares foram congestão, edema intersticial, enfisemas alveolar e intersticial difusos, áreas de hepatização vermelha em lobo acessório e infiltrado neutrofílico em pleura e parênquima com congestão difusa severa, tendo como causa mortis a insuficiência cardiorrespiratória. O histórico de problemas respiratórios e o tipo de sistema de criação da propriedade foram importantes para o diagnóstico. No entanto, apesar do quadro avançado, dificuldade terapêutica e com o isolamento do agente, os achados clínicos e exames complementares, contribuíram para diagnóstico presuntivo, porém a pleuropneumonia intersticial aguda severa foi confirmada pelos achados anatomopatológicos.

**Palavras-chave:** Complexo respiratório bovino, dispneia, pneumonia, confinamento



## SEVERE ACUTE INTERSTITIAL PLEUROPNEUMONIA IN GIROLANDO COW: CASE REPORT

Respiratory diseases have great transmission capacity, especially in confinement systems, among the pneumonias, the most frequent are bronchopneumonia and interstitial pneumonia, which in turn cause lung inflammation due to viral infections or inhalation of toxins. The objective is to report a case of interstitial pleuropneumonia in a five-year-old pregnant Girolando cow, from the Compost Barn of Jerônimo Monteiro/ES, treated at the Production Animal Sector of the Veterinary Hospital of the Federal University of Espírito Santo, Campus Alegre/ES. In the anamnesis she reported other cases of pneumonia in different categories of animals. Clinically, apathy, anorexia, dehydration and enophthalmos were observed. Severe respiratory symptoms showed mixed dyspnea with areas of muffledness and crepitus on pulmonary auscultation, with increased field on right chest percussion, in addition to increased dorsal volume showing subcutaneous emphysema that extended from the dorsal cervical region to the thoracolumbar region. Ultrasound examination revealed the presence of a large amount of gas, especially in the right hemithorax, associated with the presence of a moderate amount of anechogenic content interspersed in the lung lobes. The blood count revealed hyperfibrinogenemia, mild, regenerative left nuclear shift of neutrophils, as well as toxic neutrophils with donut-shaped nuclei. In biochemistry, there was an increase in serum concentrations of total protein, globulin and urea, in addition to hypoalbuminemia. Despite the treatment, there



was a decline in the clinical condition of the animal that died the next day. The pulmonary anatomopathological findings were congestion, interstitial edema, diffuse alveolar and interstitial emphysema, areas of red hepatization in the accessory lobe, and neutrophilic infiltrate in the pleura and parenchyma with severe diffuse congestion, whose cause of death was cardiorespiratory failure. The history of respiratory problems and the type of rearing system on the property were important for the diagnosis. However, despite the advanced condition, therapeutic difficulty and the isolation of the agent, the clinical findings and complementary exams contributed to a presumptive diagnosis, but severe acute interstitial pleuropneumonia was confirmed by the anatomopathological findings.

**Keywords:** Bovine respiratory complex, dyspnea, pneumonia, confinement



## **SURTO DE PNEUMONIA PROVOCADA POR *Pasteurella multocida* EM BEZERROS NO SEMIÁRIDO DO RIO GRANDE DO NORTE**

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O complexo respiratório bovino (CBR) é responsável por altas taxas de morbimortalidade, principalmente, nos rebanhos confinados da espécie. Os surtos já relatados têm caráter multifatorial e estão associados a vários patógenos, dentre eles, a *Pasteurella multocida*, uma bactéria gram-negativa oportunista que coloniza o trato respiratório dos bovinos. Contudo, nos últimos anos, relatos de casos fatais dessa enfermidade respiratória, ocasionados por esse agente, vêm crescendo em bovinos. Nesse contexto, foi acompanhado um surto respiratório em bezerros mestiços da raça Pardo-Suíço com idade e peso variados. Os sinais clínicos observados foram: anorexia, febre, dispneia, corrimento nasal mucopurulento, perda de peso e tosse produtiva. No



levantamento epidemiológico constatou-se que cerca de 10 dias antes do aparecimento dos sinais clínicos, foram introduzidos no rebanho seis bovinos adquiridos em uma feira agropecuária, sem a realização de quarentena. Dos 38 bezerros que compunham o rebanho, 29 adoeceram e 25 morreram, desses últimos, três foram submetidos à necropsia. Macroscopicamente observou-se pneumonia fibrinopurulenta, presença de secreção mucopurulenta no interior dos brônquios e bronquíolos e múltiplos abscessos nos lobos pulmonares. Fragmentos de pulmão coletados para exame histológico revelaram a presença de neutrófilos, monócitos e macrófagos, além de fibrina e detritos celulares no lúmen dos brônquios, bronquíolos e alvéolos, associado a miríades bacterianas intralesionais. Ainda foram coletadas amostras da secreção bronquial para cultivo bacteriano, havendo o crescimento de *P. multocida* cujo antibiograma revelou a sensibilidade da bactéria a um pentabiótico. Repassando-se ao proprietário a terapia adequada, a mortalidade cessou. A formação de lotes de bezerros com animais de origens distintas, desconhecendo os aspectos sanidade ou situação vacinal dos rebanhos, é um exemplo de fator estressante que torna os animais susceptíveis ao contágio da CRB. Portando, corrigir esses fatores predisponentes, é a forma de prevenir e evitar a perpetuação da doença nos rebanhos.

**Palavras-chave:** febre do transporte; bactéria; terneiro



## **OUTBREAK OF PNEUMONIA CAUSED BY *Pasteurella multocida* IN CALVES IN THE SEMI-ARID OF RIO GRANDE DO NORTE**

The bovine respiratory complex (CBR) is responsible for high rates of morbidity and mortality, especially in confined herds of the species. The outbreaks already reported have a multifactorial character and are associated with several pathogens, including *Pasteurella multocida*, an opportunistic gram-negative bacterium that colonizes the respiratory tract of cattle. However, in recent years, reports of fatal cases of this respiratory disease caused by this agent have been increasing in cattle. In this context, a respiratory outbreak was observed in crossbred calves of the Brown-Swiss breed of varying age and weight. The clinical signs observed were: anorexia, fever, dyspnea, mucopurulent nasal discharge, weight loss and productive cough. In the epidemiological survey, it was found that about 10 days before the appearance of clinical signs, six cattle acquired at an agricultural fair were introduced into the herd, without quarantine. Of the 38 calves that made up the herd, 29 became ill and 25 died, of the latter, three were submitted to necropsy. Macroscopically, it was observed fibrinopurulent pneumonia, presence of mucopurulent secretion inside the bronchi and bronchioles and multiple abscesses in the lung lobes. Lung fragments collected for histological examination revealed the presence of neutrophils, monocytes and macrophages, as well as fibrin and cellular debris in the lumen of the bronchi, bronchioles and alveoli, associated with myriad





intralesional bacteria. Samples of bronchial secretions were also collected for bacterial culture, with the growth of *P. multocida* whose antibiogram revealed the sensitivity of the bacteria to a pentabiotic. By providing the owner with adequate therapy, mortality ceased. The formation of batches of calves with animals from different origins, ignoring the health aspects or vaccination status of the herds, is an example of a stressful factor that makes animals susceptible to CRB contagion. Therefore, correcting these predisposing factors is the way to prevent and avoid the perpetuation of the disease in herds.

**Keywords:** transport fever; bacterium; calf



## **SURTO DE RINOTRAQUEÍTE INFECCIOSA EM BOVINOS DE CORTE CRIADOS NO RECÔNCAVO DA BAHIA – RELATO DE CASO**

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As infecções por Herpesvírus bovino tipo 1 estão frequentemente associadas a casos de rinotraqueíte infecciosa bovina (IBR), ainda que outras manifestações clínicas também sejam observadas. A infecção pode ocorrer por contato íntimo entre mucosas ou por contato destas superfícies com aerossóis e secreções corpóreas. Os animais infectados tornam-se portadores do vírus por toda vida e a infecção latente pode ser reativada frente a fatores de estresse. Relata-se um surto de IBR em rebanho bovino de corte, criados no Estado da Bahia, com morbidade de 30% e letalidade de 15%. A doença foi identificada em 13 animais que pertenciam a um lote de 43 bovinos da raça Nelore, de 18 – 24 meses, que apresentavam apatia e corrimento nasal, 10 dias após transporte. O colaborador da fazenda também informou que dois animais exibiram piora do quadro os quais vieram a óbito. Durante o exame físico foram identificados apatia, inapetência, desidratação moderada, mucosas oculopalpebrais e nasal avermelhadas,



lacrimejamento, linfonodos mandibulares aumentados de tamanho e febre. Alguns animais exibiam corrimento nasal bilateral seromucoso com discreto aumento de volume de narinas e dispneia inspiratória. Um bovino apresentava depressão, estertor respiratório, secreção mucopurulenta fétida e profusa, e lesões erosivas circulares em mucosa nasal e oral. Foram colhidas amostras de sangue de 6 animais com pior quadro clínico para realização de hemograma e diagnóstico sorológico pareado por ELISA indireto com Kits comerciais (Bovine Rhinotracheitis virus antibodytest kit, HerdChek®, IDEXX Laboratories, EUA). O exame hematológico indicou leucocitose por linfocitose na maioria dos animais e hiperfibrinogenemia com leucopenia intensas no bovino que exibiu pior quadro clínico. O exame sorológico apontou soroconversão nos animais avaliados com elevação intensa dos títulos de anticorpos. Os bovinos com sinais clínicos foram segregados e foi realizado tratamento sintomático com antipiréticos, anti-inflamatórios, hidratação e antibioticoterapia. O bovino com sinais clínicos mais severos também foi tratado com mucolítico, nutracêuticos e curativos nas erosões. Os dados clínicos e epidemiológicos puderam direcionar os exames complementares adequados, que auxiliaram no diagnóstico da IBR por Herpesvírus bovino devido ao aumento da titulação de anticorpos. Após o controle das manifestações da doença no lote, foi recomendada a vacinação dos animais.

**Palavras-chave:** Doença respiratória, Herpesvírus bovino tipo 1, hematologia, sorologia, ELISA indireto



## **OUTBREAK OF INFECTIOUS RHINOTRACHEITIS IN BEEF CATTLE BREEDED IN THE STATE OF BAHIA, BRAZIL – CASE REPORT**

Bovine herpesvirus type 1 infections are frequently associated with cases of infectious bovine rhinotracheitis (IBR), although other clinical manifestations are also observed. Infection can occur by intimate contact between mucous membranes or by contact of these surfaces with aerosols and bodily secretions. Infected animals become carriers of the virus for life and the latent infection can be reactivated by stress factors. An outbreak of IBR is reported in beef cattle, bred in the State of Bahia, with a morbidity of 30% and a lethality of 15%. The disease was identified in 13 animals that belonged to a lot of 43 Nelore cattle, aged 18 – 24 months, which presented apathy and nasal discharge, 10 days after transport. The farm employee also reported that two animals showed worsening of the condition and died. During the physical examination, apathy, inappetence, moderate dehydration, reddish oculopalpebral and nasal mucosa, tearing, enlarged mandibular lymph nodes and fever were identified. Some animals had bilateral seromucous nasal discharge with a slight increase in nasal volume and inspiratory dyspnea. One bovine presented depression, snore, fetid and profuse mucopurulent secretion, and circular erosive lesions in the nasal and oral mucosa. Blood samples were collected from 6 animals with the worst clinical condition for hematological exams and serological diagnosis paired by indirect ELISA with Bovine Rhinotracheitis virus antibodytest kit, HerdChek®, IDEXX



Laboratories, USA. The hematological examination indicated leukocytosis by lymphocytosis in most animals and hyperfibrinogenemia with intense leukopenia in the bovine with the worst clinical condition. The serological examination showed seroconversion in the evaluated animals with intense elevation of antibody titers. Cattle with clinical signs were segregated and symptomatic treatment with antipyretics, anti-inflammatory drugs, hydration and antibiotic therapy was performed. The bovine with more severe clinical signs was treated with mucolytics, nutraceuticals and erosion dressings. Clinical and epidemiological data could direct the appropriate complementary exams, which helped in the diagnosis of IBR by bovine herpesvirus due to the increase in antibody titration. After controlling the manifestations of the disease in the flock, vaccination of the animals was recommended.

**Keywords:** Respiratory disease, bovine herpesvirus type 1, hematology, serology, indirect ELISA



## ULTRASSONOGRAFIA TORÁCICA NO DIAGNÓSTICO DE DOENÇAS RESPIRATÓRIAS EM BOVINOS: ESTUDO RETROSPECTIVO DE 132 CASOS

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As Afecções respiratórias são comuns na clínica médica de ruminantes, sobretudo em animais criados de forma intensiva e bezerros, e causam grandes prejuízos à pecuária. Entretanto, a percepção clínica na fase inicial constitui um grande desafio. Portanto, o exame clínico detalhado é fundamental para identificar as afecções respiratórias e diferenciá-las de outras doenças que afetam os demais sistemas orgânicos. Contudo, o exame clínico não é capaz de estabelecer o tipo e extensão das lesões no sistema respiratório inferior, além disso alguns animais podem não apresentar sintomatologia clínica – subclínicos, sendo imprescindível o uso de exames complementares para confirmação/exclusão diagnóstica. A ultrassonografia transtorácica destaca-se por ser uma técnica não invasiva, sem efeitos colaterais, com custo operacional baixo, com alta acurácia para na detecção, caracterização e quantificação de



lesões pulmonares suspeitadas na avaliação clínica. Neste contexto, este trabalho tem como objetivo destacar a importância do exame ultrassonográfico como ferramenta diagnóstica em bovinos acometidos de afecções respiratórias atendidos na Clínica de Bovinos de Garanhuns, Campus da Universidade Federal Rural de Pernambuco (CBG/UFRPE). De janeiro a dezembro de 2019, foram realizados 132 exames ultrassonográficos de tórax. As imagens encontradas nestes exames sugeriam os seguintes diagnósticos: enfisema pulmonar sem outras alterações (43/132 [32,57%]); broncopneumonias abscedativas (37/132 [28,03%]); broncopneumonias não supurativas (17/132 [12,87%]); pneumonias abscedativas (10/132 [7,57%]); pleurite (8/132 [6,06%]); pneumonias granulomatosas/nodulares (7/132 [5,3%]); pneumonias (7/132 [5,30%]); pleuropneumonias (3/132 [2,27%]). Em relação às lesões, as mais comumente encontradas foram enfisema pulmonar (53/132) e lesões abscedativas (47/132), enquanto a consolidação pulmonar não associada à lesões granulomatosas/piogranulomatosas foi identificada em apenas 12 exames, além disso a efusão pleural foi vista em apenas 6 exames. A ultrassonografia aplicada na rotina hospitalar constitui uma maneira ágil e concisa para se estabelecer o diagnóstico, o prognóstico e a conduta clínica, reduzindo assim custos desnecessários ao produtor em casos avançados de determinadas enfermidades, nos quais não haverá a resolução clínica e/ou o retorno da produtividade do animal. Os levantamentos retrospectivos são importantes ferramentas para avaliar a frequência de determinadas afecções e permitir comparações entre diferentes estudos.

**Palavras-chave:** Diagnóstico por Imagem, Pneumonia, Ruminantes



## **THORACIC ULTRASOUND IN THE DIAGNOSIS OF RESPIRATORY DISEASES IN CATTLE: A RETROSPECTIVE STUDY OF 132 CASES**

Respiratory disorders are common in ruminant medical clinics, especially in intensively raised animals and calves, and cause great damage to livestock. However, clinical perception in the initial phase is a major challenge. Therefore, a detailed clinical examination is essential to identify respiratory disorders and differentiate them from other diseases that affect other organ systems. However, the clinical examination is not able to establish the type and extent of lesions in the lower respiratory system, in addition, some animals may not present clinical - subclinical symptoms, and the use of complementary tests for diagnostic confirmation / exclusion is essential. Transthoracic ultrasound stands out for being a non-invasive technique, without side effects, with low operating cost, with high accuracy for the detection, characterization and quantification of suspected lung lesions in the clinical evaluation. In this context, this study aims to highlight the importance of ultrasound as a diagnostic tool in cattle with respiratory disorders treated at the Clínica de Bovinos de Garanhuns, Campus of the Universidade Federal Rural de Pernambuco (CBG/UFRPE). From January to December 2019, 132 chest ultrasound examinations were performed. The images found in these exams suggested the following diagnoses: pulmonary emphysema without other alterations (43/132 [32.57%]); Abscessive





bronchopneumonia (37/132 [28.03%]); non-suppurative bronchopneumonia (17/132 [12.87%]); abscessive pneumonias (10/132 [7.57%]); pleuritis (8/132 [6.06%]); granulomatous/nodular pneumonias (7/132 [5.3%]); pneumonias (7/132 [5.30%]); pleuropneumonia (3/132 [2.27] %) Regarding the lesions, the most commonly found were pulmonary emphysema (53/132) and abscessive lesions (47/132), while pulmonary consolidation not associated with granulomatous/pyogranulomatous lesions was identified in only 12 exams, in addition to pleural effusion was seen in only 6 exams. Ultrasonography applied in hospital routine is an agile and concise way to establish the diagnosis, prognosis and clinical management, thus reducing unnecessary costs to the producer in advanced cases of certain diseases, in which there will be no clinical resolution and/or a return to the animal's productivity. Retrospective surveys are important tools to assess the frequency of certain conditions and allow comparisons between different studies.

**Keywords:** Diagnostic Imaging, Pneumonia, Ruminants



ÁREA TEMÁTICA:

# SANIDADE GERAL DOS BOVINOS



## ADENOCARCINOMA DE GLÂNDULA SALIVAR COM METÁSTASE PULMONAR EM VACA: RELATO DE CASO

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Os tumores das glândulas salivares (TGS) fazem parte de um grupo heterogêneo de neoplasias cujo aspecto morfológico complexo dificulta o diagnóstico. Esses tumores possuem ocorrência rara em animais, embora haja casos descritos em várias espécies. No entanto, em bovinos são considerados tumores malignos de ocorrência rara. Foi atendida no HOVET/UFERSA uma fêmea bovina, Guzerá, 10 anos, 290 Kg, com histórico de aumento de volume no antímero direito da face. No exame físico, além do aumento de volume de consistência firme, observou-se rotação de cabeça, ptose palpebral, labial e auricular no lado direito e epífora acentuada no olho direito. Após 5 dias de internamento, o animal apresentou piora clínica considerável, optando-se pela eutanásia e encaminhamento para o exame necroscópico. Macroscopicamente, evidenciou-se úlceras de abomaso do tipo II; áreas granulomatosas multifocais no pulmão; linfonodos mediastínicos aumentados com pontos de aspecto granulomatoso; e massa de consistência firme na região parotídea do antímero direito da face. Fragmentos da glândula parótida e dos pulmões foram enviados para exame



histopatológico, onde observaram-se células neoplásicas epiteliais, arranjadas em ninhos e pacotes, densamente agrupadas e sustentadas por um estroma fibroso. As células também apresentavam pleomorfismo moderado a elevado, com anisocitose e eventual cariomegalia, observando-se ainda figuras de mitose atípicas e focos de necrose. Esses achados confirmaram o diagnóstico de adenocarcinoma de glândula salivar com metástase pulmonar. Os TGS acometem principalmente animais mais velhos e dentro da produção animal os indivíduos mais susceptíveis são aqueles destinados à produção de leite ou reprodutores, categorias que favorecem a permanência desses animais por mais tempo nos rebanhos, como observado nesse relato de caso. Portanto, é importante diagnóstico diferencial para patologias que levam ao aumento de volume na cabeça, linfadenopatia, disfagia, perda de peso progressiva e epífora, principalmente nos animais de produção, tendo como ferramentas os exames necroscópico e histopatológico.

**Palavras-chaves:** neoplasia maligna; bovinos; semiárido



## **SALIVARY GLAND ADENOCARCINOMA WITH LUNG METASTASIS IN A COW: CASE REPORT**

Salivary gland tumors (SGT) are an important and heterogeneous group of neoplasms whose complex morphological aspect makes diagnosis difficult. These tumors are rare in animals, although there are cases described in several species. In cattle, they are considered rare malignant tumors. A female bovine Guzerá, 10 years old, 290 kg, with a history of swelling on the right side of the face, was seen at HOVET/UFERSA. On physical exam, besides the firm consistency swelling, rotation of the head, palpebral, labial and auricular ptosis on the right side and accentuated epiphora in the right eye were observed. After 5 days of hospitalization, the animal presented considerable clinical worsening, and the animal was euthanized and sent for necroscopic examination. Macroscopic examination revealed type II abomasal ulcers; multifocal granulomatous lesions in the lung; enlarged mediastinal lymph nodes with granulomatous spots; and a firm consistency in the parotid region of the right antimer of the face. Fragments of the parotid gland and lungs were sent for histopathological examination, and important neoplastic cells were seen, arranged in nests and bundles, densely packed and supported by a fibrous stroma. The cells also showed moderate to high pleomorphism, with anisocytosis and eventual karyomegaly; atypical mitosis figures and foci of necrosis were also observed. These findings confirmed the diagnosis of parotid gland adenocarcinoma with lung metastasis. SGT mainly affect older animals and, within animal production, the most susceptible individuals are those intended for



milk production or breeding, categories that favor the permanence of these animals for longer in the herd, as observed in this case report. Therefore, it is important to make a differential diagnosis for pathologies that lead to head swelling, lymphadenopathy, dysphagia, progressive weight loss and epiphora, especially in production animals, using necroscopic and histopathological exams as tools.

**Keywords:** malignant neoplasm; cattle; semiarid



## ALTERAÇÕES NO LEUCOGRAMA E PROTEINOGRAMA CONFORME A GRAVIDADE E TEMPO DE EVOLUÇÃO DA MASTITE CLÍNICA EM VACAS LEITEIRAS

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A mastite clínica tem grande importância na bovinocultura de leite pela alta prevalência e impacto econômico, e exames complementares como leucograma e proteinograma podem auxiliar na avaliação clínica das vacas com mastite. Diante disso, o objetivo deste trabalho foi comparar as alterações no leucograma e proteinograma de vacas com mastite conforme a gravidade e o tempo de evolução do quadro clínico. Foram avaliadas 49 vacas leiteiras com mastite clínica, antes do tratamento, foi coletado sangue em tubo com EDTA (2ml) para avaliação do leucograma e mensuração do fibrinogênio e em tubo seco (8ml), obtendo soro para mensuração da concentração de proteínas totais, albumina e globulinas (calculada). Os animais foram classificados em grupos pelo tempo de evolução, sendo evolução curta (G<sub>curt</sub>, n=30) com menos de dois dias ou evolução longa (G<sub>lon</sub>, n=19) mais de dois dias com mastite, e classificados pela gravidade, em vacas com manifestações sistêmicas (G<sub>grav</sub>, n=19), como febre, anorexia, desidratação intensa e/ou decúbito (maior gravidade) ou vacas sem manifestações sistêmicas (G<sub>ñgr</sub>, n=30), menor gravidade, apenas com alterações em leite e glândula mamária. A comparação entre grupos foi por Teste t não pareado (paramétricos) ou teste de *Mann Whitney* (não paramétricos)



( $p < 0,05$ ). Na comparação entre gravidade da mastite houve diferença para número de metamielócitos: GÑgr  $153 \pm 633$  clls/ $\mu$ L e Ggrav  $370 \pm 613$  clls/ $\mu$ L; e para neutrófilos bastonetes: GÑgr  $231 \pm 240$  clls/ $\mu$ L e Ggrav  $1094 \pm 1236$  clls/ $\mu$ L. Na comparação entre tempo de evolução houve diferença para leucócitos totais: GLon  $16.000 \pm 6162$  clls/ $\mu$ L e GCurt  $8.450 \pm 4100$  clls/ $\mu$ L; neutrófilos segmentados: GLon  $7.357 \pm 1163$  clls/ $\mu$ L e GCurt  $2.882 \pm 451$  clls/ $\mu$ L; albumina: GLon  $2,77 \pm 0,14$  g/dL e GCurt  $3,34 \pm 0,11$  g/dL; e para globulinas: GLon  $5,3 \pm 0,45$  g/dL e GCurt  $3,8 \pm 0,17$  g/dL. Quadros graves, com manifestações sistêmicas, causaram desvio a esquerda mais intenso. Quadros com evolução mais longa tiveram aumento do número de leucócitos totais, principalmente neutrófilos segmentados, uma resposta medular ao estímulo inflamatório, além disso, neste quadro, as vacas apresentaram menores concentrações de albumina, uma proteína de fase aguda negativa e maiores concentrações de globulinas. Assim, conclui-se que existem importantes diferenças e alterações no leucograma e proteinograma conforme a gravidade e tempo de evolução da mastite em vacas leiteiras.

**Palavras-chave:** Desvio à esquerda, Leucocitose, Neutrofilia, Resposta inflamatória





## **CHANGES IN LEUKOGRAM AND PROTEINOGRAM ACCORDING TO THE SEVERITY AND TIME OF EVOLUTION OF CLINICAL MASTITIS IN DAIRY COWS**

Clinical mastitis has great importance in dairy cattle due to its high prevalence and economic impact, and complementary tests such as leukogram and proteinogram can help in the clinical evaluation of cows with mastitis. Therefore, the objective of this study was to compare the changes in the leukogram and proteinogram of cows with mastitis according to the severity and time of evolution of the clinical condition. Were evaluated 49 dairy cows with clinical mastitis, before treatment, blood was collected in a tube with EDTA (2ml) for evaluation of leukogram and measurement of fibrinogen and in a dry tube (8ml), obtaining serum for measurement of total proteins concentration, albumin and globulins (calculated). The animals were classified into groups by the time of evolution, being short evolution (GShrt, n=30) with less than two days, or long evolution (GLon, n=19) more than two days with mastitis, and classified by severity, in cows with systemic manifestations (GGrav, n=19), such as fever, anorexia, intense dehydration and/or recumbency (greater severity) or cows without systemic manifestations (GÑgr, n=30), less severe, only with changes in milk and mammary gland. Comparison between groups was performed using the unpaired t test (parametric) or the Mann Whitney test (nonparametric) ( $p < 0.05$ ). When comparing the severity of mastitis, there was a difference for number of metamyelocytes: GÑgr  $153 \pm 633$  clls/ $\mu$ L and GGrav  $370 \pm 613$  clls/ $\mu$ L; and for band



neutrophils:  $G\tilde{N}gr$   $231\pm 240$  clls/ $\mu$ L and  $GGrav$   $1094\pm 1236$  clls/ $\mu$ L. When comparing evolution time, there was a difference for total leukocytes:  $GLon$   $16,000\pm 6,162$  clls/ $\mu$ L and  $GShrt$   $8,450\pm 4,100$  clls/ $\mu$ L; segmented neutrophils:  $GLon$   $7,357\pm 1,163$  clls/ $\mu$ L and  $GShrt$   $2,882\pm 451$  clls/ $\mu$ L; albumin:  $GLon$   $2.77\pm 0.14$  g/dL and  $GShrt$   $3.34\pm 0.11$  g/dL; and for globulins:  $GLon$   $5.3\pm 0.45$  g/dL and  $GShrt$   $3.8\pm 0.17$  g/dL. Severe conditions, with systemic manifestations, caused more intense left shift. In cases with longer evolution there was an increase in the number of total leukocytes, mainly segmented neutrophils, a medullary response to the inflammatory stimulus, moreover, in this case, cows had lower concentrations of albumin, a negative acute phase protein, and higher concentrations of globulins. Thus, it's concluded that there are important differences and changes in the leukogram and proteinogram according to the severity and time of evolution of mastitis in dairy cows.

**Keywords:** Inflammatory response, Left shift, Leukocytosis, Neutrophilia



## **ATRESIA ANAL ASSOCIADO À FÍSTULA RETROVAGINAL EM BEZERRA MESTIÇA: RELATO DE CASO**

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As anomalias congênitas são malformações estruturais ou funcionais que ocorrem durante a embriogênese, sendo a atresia anal uma das alterações congênitas descritas em neonatos e pode ser classificada em quatro tipos de acordo com os graus de disgenesia ou agenesia do reto e do ânus. Portanto, os casos de atresia anal podem estar associados com outras malformações congênitas, que consistem na formação incompleta de um órgão, como na aplasia da cauda. Objetiva-se relatar um caso atendido no Hospital Veterinário de atresia anal associado à fístula retovaginal em bezerra mestiça (Gir x Guzerá), oriunda de parto eutócico, com dois dias de idade, pesando 27 kg. Na anamnese relatou-se a eliminação das fezes e urina pela vagina e a ausência da cauda, logo após o nascimento. Clinicamente, o animal apresentava ausência tanto do orifício anal como da cauda, além da presença de fezes na região vulvar e os demais parâmetros clínicos estavam fisiológicos para a espécie. Os resultados do hemograma e bioquímico séricos estavam dentro dos valores de referência para a espécie. Ao exame radiográfico, com a injeção de contraste positivo a base de iohexol (300 mg I/mL), evidenciou-se retenção fecal, agenesia das



vértebras sacrococcígea e caudais além da comunicação retal com a abertura do vestíbulo vaginal. Utilizou-se a técnica cirúrgica de anuplastia associada ao reparo da fístula retovaginal como tratamento. Algumas alterações congênitas não são compatíveis com a vida e inviáveis para a correção cirúrgica, ainda há casos em que essas alterações são identificadas apenas na morte ou abate do animal. Logo, classificou-se a atresia anal desse caso como grau três devido a formação de fundo cego no reto proximal e a ausência de ânus desenvolvido. O diagnóstico das alterações congênitas geralmente é realizado através do histórico, achado clínico-epidemiológico logo após o nascimento, sendo confirmado com exames de imagem e anatomopatológicos. Conclui-se que o caso de atresia anal associado à fístula retovaginal com o diagnóstico clínico e correção cirúrgica precoces foram fundamentais para o bom prognóstico do animal.

**Palavras-chave:** Monstros fetais, malformações congênitas, anuplastia, agenesia, neonato



## **ANAL ATRESIA ASSOCIATED WITH RECTROVAGINAL FISTULA IN A CROSSBREED CALF: CASE REPORT**

Congenital anomalies are structural or functional malformations that occur during embryogenesis, and anal atresia is one of the congenital alterations described in neonates and can be classified into four types according to the degrees of dysgenesis or agenesis of the rectum and anus. Therefore, cases of anal atresia may be associated with other congenital malformations, which consist of incomplete formation of an organ, such as tail aplasia. The objective is to report a case treated at the Veterinary Hospital of anal atresia associated with rectovaginal fistula in a crossbred heifer (Gir x Guzerá), from eutocic delivery, with two days of age, weighing 27 kg. The anamnesis reported the elimination of feces and urine through the vagina and the absence of the tail, soon after birth. Clinically, the animal had the absence of both the anal orifice and the tail, in addition to the presence of feces in the vulvar region, and the other clinical parameters were physiological for the species. The results of the blood count and serum biochemistry were within the reference values for the species. The radiographic examination, with the injection of positive contrast based on iohexol (300 mg I/mL), showed fecal retention, agenesis of the sacrococcygeal and caudal vertebrae, in addition to rectal communication with the opening of the vaginal vestibule. The surgical technique of anuplasty associated with the repair of the rectovaginal fistula was used as treatment. Some congenital alterations are not compatible with life and unfeasible for surgical correction, there are still cases in which these alterations are only identified in the death or slaughter of the



animal. Therefore, the anal atresia in this case was classified as grade three due to the formation of a blind bottom in the proximal rectum and the absence of a developed anus. The diagnosis of congenital alterations is usually performed through the history, clinical-epidemiological finding soon after birth, being confirmed with imaging and anatomopathological exams. It is concluded that the case of anal atresia associated with rectovaginal fistula with early clinical diagnosis and surgical correction were fundamental for the good prognosis of the animal.

**Keywords:** Fetal monsters, congenital malformations, anuplasty, agenesis, neonate



## **AVALIAÇÃO DA IMUNIDADE EM VACAS HOLANDESAS SUPLEMENTADAS COM MINERAIS DURANTE O PERÍODO DE TRANSIÇÃO**

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O período de transição é o mais desafiador e crítico devido às doenças metabólicas e aumento da desregulação imunológica. A mobilização excessiva do tecido adiposo gera altas concentrações de ácidos graxos não esterificados e beta-hidroxibutirato quando associada à hipoglicemia, contribui para a desregulação imunológica periparturiente. O objetivo foi avaliar a influência da suplementação de minerais injetáveis em relação ao perfil inflamatório e dinâmica de IgG em vacas Holandesas durante o período de transição. O estudo foi realizado em um laticínio comercial com produção média diária de leite de 35L/animal, em São Pedro, São Paulo, Brasil. Dieta e pré-mistura formuladas de acordo com o NRC. Vacas saudáveis foram incluídas 30 dias antes do parto e alocadas para controle (C; n=32) ou grupo de tratamento (T; n=34). As vacas tratadas receberam três injeções (10 mL) de suplemento mineral contendo 575,12 mg de P; 31,36 mg K; 29,88 mg Mg; 14,91 mg Cu; 10,89 mg Se, por via intramuscular, 30 a 20 dias pré-parto, ao parto e 15 dias pós-parto. As amostras



foram colhidas: três semanas antes do parto (M-3); duas semanas (M-2); uma semana (M-1); na semana do parto (M0); uma semana pós-parto (M+1); duas semanas (M+2) e três semanas (M+3). Tubo de vácuo com ativador de coágulo foi usado para obter soro sanguíneo. A medição de IgG no soro foi realizada por ensaio ELISA interno, enquanto a concentração de haptoglobina foi determinada por sua capacidade de se ligar à hemoglobina usando espectrofotometria. A suplementação mineral injetável teve efeito na concentração de IgG (mg/mL) na 1ª lactação (C=27,19±1,53 vs T= 33,32±1,96; p=0,0044) e com ≥3 lactações (C=30,07±1,98 vs T=37,40± 2,08; p=0,0183). Efeitos na concentração de haptoglobina (mg/mL) foram observados na 2ª lactação (C=5,84±0,48 vs T=4,3±0,48; p=0,0234). Para vacas multíparas, foram observados efeitos de IgG (C=28,09±1,45 vs T= 36,87±1,69; p=0,0027) e haptoglobina (C=6,15±0,65 vs T= 4,32±0,3; p=0,0296). Resultados semelhantes também foram observados para o número total de animais para IgG (C=27,68±1,05 vs T=35,63±1,3; p=<0,0001) e haptoglobina (C=5,84±0,42 vs T=4,21±0,23; p= 0,0518). No entanto, não foi possível observar o efeito na comparação entre primíparas (P) e multíparas (M) (P=29,81±1,23 vs M=32,83±1,16; p=0,3865). Os resultados obtidos neste estudo sugerem que o protocolo de minerais injetáveis utilizado, melhora significativamente a imunidade e o perfil pró-inflamatório em vacas Holandesas de alta produção.

**Palavras-chave:** minerais, período de transição, IgG, haptoglobina, imunidade





## IMMUNITY EVALUATION IN HOLSTEIN COWS SUPPLEMENTED WITH MINERALS DURING THE TRANSITION PERIOD

The transition period is the most challenging and critical due to metabolic diseases and immune dysregulation increase. Adipose tissue excessive mobilization generates high concentrations of non-esterified-fatty-acids and beta-hydroxybutyrate when associated with hypoglycemia, contributes to periparturient immune dysregulation. The objective was evaluate injectable mineral supplementation influence in relation to inflammatory profile and IgG dynamics in Holstein cows during the transition period. The study carried out in a commercial dairy operation with an average daily milk production 35L/animal, at São Pedro, São Paulo, Brazil. Diet and premix formulated according to the NRC. Healthy cows were included 30 days before calving, and allocated to control (C; n=32) or treatment group (T; n=34). Treated cows received three injections (10 mL) of a mineral supplement containing 575.12 mg P; 31.36 mg K; 29.88 mg Mg; 14.91 mg Cu; 10.89 mg Se, intramuscularly 30 to 20 days prepartum, at calving and 15 days postpartum. Samples was harvest: three weeks before delivery (M-3); two weeks (M-2); one week (M-1); at calving week (M0); one week postpartum (M+1); two weeks (M+2) and three weeks (M+3). Vacuum tube with clot activator was used to obtain blood serum. Serum IgG measurement was performed by in-house ELISA assay, while haptoglobin concentration was determined by its ability to bind hemoglobin using spectrophotometry. Injectable mineral supplementation had an effect on IgG concentration (mg/mL) for 1<sup>st</sup> lactation (C=27.19±1.53 vs T=



33.32±1.96; p=0.0044) and with ≥3 lactations (C=30.07± 1.98 vs T=37.40±2.08; p=0.0183). Effects on haptoglobin concentration (mg/mL) were observed for 2<sup>nd</sup> lactation (C=5.84±0.48 vs T=4.3±0.48; p=0.0234). For multiparous cows, both IgG (C=28.09±1.45 vs T= 36.87±1.69; p=0.0027) and haptoglobin (C=6.15±0.65 vs T= 4.32±0.3; p=0.0296) effects were observed. Similar results were also observed for the total number of animals for both IgG (C=27.68±1.05 vs T=35.63±1.3; p=<.0001) and haptoglobin (C=5.84±0.42 vs T=4.21± 0.23; p=0.0518). However, it was not possible to observe the effect in comparison between primiparous (P) and multiparous (M) (P=29.81±1.23 vs M=32.83±1.16; p=0.3865). The results obtained in this study suggest that the injectable mineral protocol used significantly improves the immunity and pro-inflammatory profile in high yielding Holstein cows.

**Keywords:** mineral, transition period, IgG, haptoglobin, immunity



## **AVALIAÇÃO DAS VARIÁVEIS CLIMATOLÓGICAS PARA VALIDAÇÃO DAS CONDIÇÕES AMBIENTAIS DAS VACAS EM LACTAÇÃO**

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A análise das condições de conforto térmico em ambientes de produção animal pode ser realizada mediante o cálculo de índices de conforto térmico e programas de computador utilizando-se *softwares* específicos de interpolação de dados para elaboração de mapas de variabilidade espacial de parâmetros meteorológicos. Assim, esse trabalho foi realizado com o objetivo de mensurar os indicadores ambientais que interferem diretamente no sistema produtivo, comportamental e fisiológico do animal. Foram criados mapas de variabilidade espacial da temperatura do ar e de índices de conforto térmico de animais, utilizando o método interpolador *IDW (Inverse Distance Weighted)*, ou Inverso da Distância Ponderada. O aplicativo Aurora foi desenvolvido por meio da linguagem de programação orientada a objetos Java, mediante o ambiente de desenvolvimento integrado *Android Studio* e os dispositivos móveis foram



criados utilizando-se sensores de baixo custo de temperatura do ar, temperatura de globo negro e umidade relativa, juntamente com microcontroladores de plataforma eletrônica de código aberto – Arduino. Foi utilizado um rebanho leiteiro. As avaliações foram realizadas considerando os mapas de temperatura do ar e dos índices de conforto térmico, criados no próprio local de coleta de dados. Os valores medidos foram comparados aos valores coletados mensalmente pelos *dataloggers* HOBO U12-012. Dados zootécnicos necessários para a análise foram coletados diretamente do programa de gerenciamento da propriedade participante. Foram criados 34 mapas, considerando os períodos de máxima e mínima, o aplicativo desenvolvido foi uma ferramenta capaz de avaliar o bem-estar de bovinos. O uso de mapas de variabilidade espacial da temperatura do ar (°C), temperatura de globo negro (°C) e umidade relativa (%), identificou na quarta semana em relação à terceira semana, elevação média de, aproximadamente, 4°C para ambos os períodos e redução de 8% da umidade relativa. A média de temperatura do ar foi de 23,6°C com umidade relativa de 53,75%. A compreensão dos fatores bioclimáticos é determinante para assegurar a produtividade animal e sanidade, uma vez que, o estresse térmico implica no aumento da frequência respiratória e cardíaca, redução da ingestão de matéria seca e o aumento do consumo de água, resultando no aumento da incidência de doenças.

**Palavras-chave:** Conforto térmico; bem-estar animal; produção de leite; lactação



## **EVALUATION OF CLIMATOLOGICAL VARIABLES TO VALIDATE THE ENVIRONMENTAL CONDITIONS OF LACTATING COWS**

The analysis of thermal comfort conditions in animal production environments can be performed by calculating thermal comfort indices and computer programs using specific data interpolation software for the elaboration of maps of spatial variability of meteorological parameters. Thus, this work was carried out with the objective of measuring the environmental indicators that directly interfere in the animal's productive, behavioral and physiological system. Maps of spatial variability of air temperature and thermal comfort indices of animals were created using the IDW (Inverse Distance Weighted) interpolator method. The Aurora application was developed using the Java object-oriented programming language, using the Android Studio integrated development environment and the mobile devices were created using low-cost sensors for air temperature, black globe temperature and relative humidity, together with open source electronic platform microcontrollers ? Arduino. A dairy herd was used. The evaluations were carried out considering the maps of air temperature and thermal comfort indices, created at the data collection site. The measured values ??were compared to the values ??collected monthly by the HOBO U12-012 data loggers. Zootechnical data required for the analysis were collected directly from the participating property management program. 34 maps were created, considering the periods of maximum and minimum, the application developed was a tool capable of evaluating the welfare of cattle. The use of maps of spatial variability of air



temperature ( $^{\circ}\text{C}$ ), black globe temperature ( $^{\circ}\text{C}$ ) and relative humidity (%), identified in the fourth week in relation to the third week, an average increase of approximately  $4^{\circ}\text{C}$  for both periods and 8% reduction in relative humidity. The average air temperature was  $23.6^{\circ}\text{C}$  with a relative humidity of 53.75%. The understanding of bioclimatic factors is crucial to ensure animal productivity and health, since heat stress implies an increase in respiratory and heart rate, reduced dry matter intake and increased water consumption, resulting in increased incidence of diseases.

**Keywords:** Thermal comfort; animal welfare; milk production; lactation



## **AVALIAÇÃO DE QUATRO CONCENTRAÇÕES DO *Allium sativum* DESIDRATADO SOBRE O *Rhipicephalus (Boophilus) microplus***

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O *Rhipicephalus (Boophilus) microplus* é um ectoparasita hematófago responsável por grandes prejuízos na bovinocultura de leite e corte no Brasil. O controle desse ectoparasita vem sendo realizado, quase que exclusivamente, com o uso de produtos químicos. A consequência desse uso desenfreado dos carrapaticidas é a multirresistência desse carrapato frente aos mais diversos princípios ativos. Dentro desse contexto, o presente estudo teve como objetivo avaliar a eficácia *in vitro* do alho em pó (*Allium sativum*) sobre a postura e taxa de eclosão de ovos de *Rhipicephalus (Boophilus) microplus*. Esse estudo foi aprovado pela Comissão de Ética no Uso de Animais – UNISEP e realizado no Laboratório de Parasitologia da Clínica Escola, entre os meses de maio e junho de 2021. Trezentas teleóginas foram coletas manualmente, de bovinos naturalmente infestados, que estavam no mínimo há quinze dias sem o contato



com qualquer tipo de carrapaticida. O delineamento experimental foi inteiramente casualizado com cinco tratamentos. O grupo controle (T0) foi tratado apenas com água deionizada e cinco níveis de concentração de *Allium sativum* em pó diluído em água deionizada: (T1) 0,5%; (T2) 1%; (T3) 2%; (T4) 4%. O teste *in vitro* foi realizado seguindo a metodologia do biocarrapaticidograma que consiste na imersão das teleóginas nas soluções testadas e posterior avaliação da taxa de postura em placa, taxa de eclosão em tubo e os cálculos do índice reprodutivo e da eficácia carrapaticida. As concentrações testadas não apresentaram efeito ( $P>0,05$ ) sobre nenhum dos parâmetros avaliados. O *Allium sativum* desidratado, nas concentrações testadas, apresentou baixa eficácia carrapaticida, com 5,29% para a concentração de 0,5%; 2,86% na concentração de 1%; 8,28% para a concentração de 2% e a maior concentração avaliada que foi de 4% apresentou uma eficácia carrapaticida de 3,39%. Considerando que a eficácia mínima de um produto deve ser de 80% para que possa ser indicado como carrapaticida, nas concentrações avaliadas no presente estudo, o *Allium sativum* desidratado não é recomendado para o controle de *Rhipicephalus (Boophilus) microplus*.

**Palavras-chave:** Carrapaticida. Carrapato bovino. Fitoterápico. Teste *in vitro*





## EVALUATION OF FOUR CONCENTRATIONS OF DEHYDRATED *Allium sativum* ON *Rhipicephalus (Boophilus) microplus*

*Rhipicephalus (Boophilus) microplus* is a hematophagous ectoparasite responsible for major losses in dairy and beef cattle farming in Brazil. The control of this ectoparasite has been realized almost exclusively with the use of chemicals. The consequence of this unrestrained use of products for tick control is the multi-resistance of this tick to various active principles. Within this context, the present study aimed to evaluate the *in vitro* efficacy of garlic powder (*Allium sativum*) on the egg laying and hatching rate of *Rhipicephalus (Boophilus) microplus*. This study was approved by the Ethics Committee on Animal Use - UNISEP and conducted in the Parasitology Laboratory of the School Clinic, between the months of May and June 2021. Three hundred teleogines were manually collected from naturally infested cattle that had not been in contact with any kind of tick remover spray for at least fifteen days. The experimental design was entirely randomized with five treatments. The control group (T0) was treated with deionized water only, and five levels of *Allium sativum* powder diluted in deionized water: (T1) 0,5%; (T2) 1%; (T3) 2%; (T4) 4%. The *in vitro* test was performed following the biocarrapaticidogram methodology, which consists in the immersion of the teleogines in the tested solutions and subsequent evaluation of the egg laying rate in a plate, hatching rate in a tube and the calculation of the reproductive index and tick efficacy. The tested concentrations showed no effect ( $P>0.05$ ) on any of the evaluated parameters. *Allium sativum* dehydrated, in the concentrations tested, showed low tick control efficacy, with 5.29% for the concentration of 0.5%; 2.86% in the concentration of 1%; 8.28% for the



concentration of 2% and the highest concentration evaluated, which was 4%, showed a tick control efficacy of 3.39%. Considering that the minimum efficacy of a product should be 80% so that it can be indicated as a tick killer, at the concentrations evaluated in this study, *Allium sativum* dehydrated is not recommended for the control of *Rhipicephalus (Boophilus) microplus*.

**Keywords:** Bovine tick, *In vitro* test. Phytotherapeutic Tick control agent



## **AVALIAÇÃO DO METABOLISMO OXIDATIVO DE NEUTRÓFILOS EM BEZERROS DA RAÇA HOLANDESA PRETA E BRANCA SUPLEMENTADOS COM PROBIÓTICO**

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Logo após o nascimento, os bezerros passam por diversos desafios estressantes, como mudanças fisiológicas tanto no quadro hematológico quanto na adaptação ao ambiente extrauterino. Probióticos são microrganismos vivos, que quando administrados em quantidades adequadas promovem benefícios a saúde do hospedeiro. Nesta fase inicial da vida, a fagocitose, realizada pelos neutrófilos, é um importante mecanismo de defesa do animal contra a invasão de microrganismos, o que resulta no aumento da produção de espécies reativas de oxigênio dessas células e este processo é fundamental na resposta imune inata. Nesse sentido, o presente estudo teve como objetivo avaliar o efeito do uso do probiótico no metabolismo oxidativo de bezerras da raça holandesa do período neonatal até o desmame (60 dias). O estudo foi aprovado no Comitê de Ética no Uso de Animais da Universidade Federal da Fronteira Sul. Vinte bezerras da raça Holandesa recém-nascidas foram selecionadas e divididas em



dois grupos. O grupo controle foi constituído por dez animais e recebeu apenas leite conforme o manejo aplicado na propriedade. Já o grupo tratamento foi constituído por dez animais, os quais receberam três gramas de probiótico administrado com a mamadeira no período matinal. Amostras de sangue foram colhidas em sete momentos desde o primeiro dia de vida (até 24 horas após o nascimento) – Momento 1, três dias – Momento 2, sete dias – Momento 3, 15 dias – Momento 4, 30 dias – Momento 5, 45 dias – Momento 6 e até 60 dias de idade – Momento 7. As amostras de sangue colhidas em tubos com anticoagulante EDTA e heparina foram utilizados para a determinação do hemograma e avaliação do metabolismo oxidativo dos neutrófilos pelo *nitroblue tetrazolium* (NBT), respectivamente. Os resultados foram analisados pelo programa GraphPad 7. Quando comparados os grupos, observou-se que os animais do grupo tratado, na maior parte dos momentos, apresentaram menor porcentagem de neutrófilos que realizaram metabolismo oxidativo do que os do grupo controle. Assim, conclui-se que o uso do probiótico foi benéfico para o metabolismo oxidativo de neutrófilos nos momentos iniciais da vida dos bezerros da raça holandesa, tornando a resposta imunológica mais eficiente.

**Palavras-chave:** Estresse Oxidativo; Neonatos; Nutrição Animal



## **EVALUATION OF THE OXIDATIVE METABOLISM OF NEUTROPHILS IN BLACK AND WHITE HOLSTEIN CALVES SUPPLEMENTED WITH PROBIOTICS**

Soon after birth, calves go through several stressful challenges, such as physiological changes both in the hematological status and in the adaptation to the extra-uterine environment. Probiotics are live microorganisms that, when administered in adequate amounts, promote host health benefits. In this initial phase of life, phagocytosis, performed by neutrophils, is an important animal defense mechanism against the invasion of microorganisms, which results in increased production of reactive oxygen species by these cells and this process is fundamental in the innate immune response. Therefore, the present study aimed to evaluate the effect of the use of probiotic on the oxidative metabolism of Holstein calves from the neonatal period until weaning (60 days). The study was approved by the Ethics Committee on Animal Use of the Universidade Federal da Fronteira Sul. Twenty newborn Holstein heifers were selected and divided into two groups. The control group consisted of ten animals and received only milk according to the management applied in the property. The treatment group consisted of ten animals, which received three grams of probiotic administered with the feeding bottle in the morning. Blood samples were collected at seven times from the first day of life (up to 24 hours after birth) - Time 1, three days - Time 2, seven days - Time 3, 15 days - Time 4, 30 days - Time 5, 45 days - Time 6, and up to 60 days of age - Time 7. Blood samples collected in tubes with EDTA and heparin anticoagulant were used for determination of



complete blood count and evaluation of neutrophil oxidative metabolism by *nitroblue tetrazolium* (NBT), respectively. The results were analyzed by GraphPad 7 software. When the groups were compared, it was observed that the animals in the treated group, in most of the times, presented a lower percentage of neutrophils that performed oxidative metabolism than those in the control group. Thus, it is concluded that the use of the probiotic was beneficial for the oxidative metabolism of neutrophils in the early stages of the life of the Holsteins calves, making the immune response more efficient.

**Keywords:** Animal Nutrition; Neonates; Oxidative Stress



## **AVALIAÇÃO QUALITATIVA DO COMPORTAMENTO DOS TRATADORES E BEZERRAS LEITEIRAS CRIADAS EM CONDIÇÕES TROPICAIS**

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O objetivo do estudo, através do uso do QBA (avaliação qualitativa do comportamento), foi interpretar a linguagem corporal de bezerras leiteiras e seus respectivos tratadores, e a partir disso avaliar a influência exercida dos comportamentos nas reações de um em contato com o outro. Foram avaliadas 20 fazendas leiteiras, localizadas no Alto Paranaíba-MG e 6 em São Paulo. A QBA dos tratadores e das bezerras foi elaborada com base nos adjetivos preconizadas pelo Welfare Quality®. A mensuração dos adjetivos foi feita através da escala visual análoga (VSA), que variou de 0 a 150 mm. Ademais, foram preenchidas fichas de caracterização do perfil de cada uma das fazendas. Os 20 adjetivos atribuídos para ambos os grupos (tratadores e bezerras) foram submetidos a análise de componentes principais (PCA). Dois foram os componentes principais identificados, um reuniu os comportamentos negativos ao bem-estar animal e o outro os comportamentos positivos. Tanto para os



tratadores quanto para as bezerras o componente principal que agrupou os adjetivos negativos teve maior variabilidade (45,33% para tratadores e 28,21% para bezerras) quando comparado aos positivos (17,97% e 14,27%, respectivamente). Na correlação de Spearman, adjetivos como relaxado, inquisitivo, agitado e sociável tiveram correlação significativa, moderada e positiva entre os grupos. Conclui-se que, os dois principais componentes extraídos dos dois grupos, tratadores e bezerras, foram capazes de espelhar padrões comportamentais semelhantes, em que os comportamentos negativos impactaram mais durante o manejo de aleitamento, e os positivos impactaram menos na variabilidade dos componentes principais, mas, apesar disso, tiveram uma correlação moderada constada entre os adjetivos nos diferentes grupos. Os achados refletem a ligação direta entre o comportamento e as reações entre humanos e animais, e confirmam a importância do manejo bem-feito no bem-estar animal.

**Palavras-chave:** Comportamento, bem-estar animal, relação homem-animal





## QUALITATIVE EVALUATION OF THE BEHAVIOR OF STOCKPERSON AND DAIRY CALVES RAISED IN TROPICAL CONDITIONS

The objective of the study, through the use of the QBA (Qualitative Behavior Assessment), was to interpret the body language of calves and their respective stockperson, and from that to evaluate the influence of behaviors on the reactions of one in contact with the other. Twenty dairy farms located in Alto Paranaíba ? MG and 6 in São Paulo were evaluated. The QBA for stockperson and calves was prepared based on the adjectives recommended by Welfare Quality®. Adjectives were measured using the visual analogue scale (VSA), which ranged from 0 to 150 mm. In addition, profile characterization sheets were filled in for each of the farms. The 20 adjectives assigned to both groups (stockperson and calves) underwent principal component analysis (PCA). Two were the main components identified, one brought together the negative behaviors to animal welfare and the other the positive behaviors. For both stockperson and calves, the main component that grouped the negative adjectives had greater variability (45.33% for stockperson and 28.21% for calves) when compared to the positive ones (17.97% and 14.27%, respectively). In Spearman's correlation, adjectives such as relaxed, inquisitive, agitated and sociable had a significant, moderate and positive correlation between the groups. It is concluded that the two main components extracted from the two groups, stockperson and calves, were able to mirror similar behavioral patterns, in which negative behaviors impacted more during lactation management, and positive ones impacted less on the variability



of the main components, but, despite this, there was a moderate correlation between the adjectives in the different groups. The findings reflect the direct link between behavior and reactions between humans and animals, and confirm the importance of good management in animal welfare.

**Keywords:** Behavior, animal welfare, human-animal relationship



## **BIOMARCADORES FISIOLÓGICOS DE BEZERROS DA RAÇA GIROLANDO CRIADOS NO TRÓPICO ÚMIDO AMAZÔNICO**

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Os cuidados sanitários com os bezerros requerem muita atenção desde o nascimento até o desmame. Para isso exames são necessários, e dessa forma faz-se obrigatório o conhecimento dos valores dos biomarcadores fisiológicos dos animais. Isto posto, objetivou-se avaliar os parâmetros clínicos de bezerros da raça girolando criados no trópico úmido amazônico. O estudo foi realizado em uma fazenda localizada no município de Paragominas, no período de dezembro de 2021 até julho de 2022, onde foram avaliados 80 bezerros sendo 28 Machos e 52 Fêmeas da raça Girolando. Os animais foram avaliados desde imediatamente ao nascimento até os 60 dias de idade, determinando-se os biomarcadores fisiológicos: frequência cardíaca (FC) e respiratória (FR) por meio de auscultação realizada com fonendoscópio de Götez (Hauptner & herberholz) e aferição da temperatura retal (TR) por meio de termômetro digital veterinário (termômetro digital veterinário Incoterm 6900.02). As médias e desvios-padrão



dos parâmetros vitais foram calculadas considerando-se diferença estatística quando  $P < 0,05$ . Do nascimento até o dia 60º dia de idade, a frequência cardíaca teve uma variação decrescente, de 141 bpm até 104 bpm; já a frequência respiratória variou de 59 mrpm a 53mrpm. A temperatura retal manteve-se estável com valores entre 38,5 e 38,9°C. Os dados observados para os biomarcadores fisiológicos de bezerros mantidos em bezerreiros tropicais nas condições edafoclimáticas do bioma Amazônico, encontram-se em consonância com aqueles descritos em outros estudos para bezerros girolandos no Piauí em 2014, que a frequência cardíaca (FC) obteve valores médios de 101 bpm, temperatura retal (TR) foi observado de 38,3°C, diferindo apenas na frequência respiratória (FR) que foi de 42 mrpm. Os valores obtidos podem ser utilizados como parâmetros fisiológicos para as funções vitais de bezerros criados nas condições mesológicas do trópico úmido amazônico.

**Palavras-chave:** bezerro, funções vitais, parâmetros fisiológicos, Amazônia



## PHYSIOLOGICAL BIOMARKERS OF GIROLANDO CALVES BREEDED IN THE AMAZON HUMID TROPIC

Health care of calves requires a lot of attention from birth to weaning. For this, exams are necessary, in that regard, it is mandatory to know the values of the physiological biomarkers of the animals. That said, the objective was to evaluate the clinical parameters of girolando calves raised in the Amazonian humid tropics. The study was carried out on a farm located in the municipality of Paragominas, from December 2021 to July 2022, where 80 calves were evaluated: 28 males and 52 females of the Girolando breed. The animals were evaluated from immediately at birth to 60 days of age, determining the biomarkers physiological parameters: heart rate (HR) and respiratory rate (RR) through auscultation performed with a Götez phonendoscope (Hauptner & herberholz) and measurement of rectal temperature (RT) using a digital veterinary thermometer (digital veterinary thermometer Incoterm 6900.02). Means and standard deviations of vital parameters were calculated considering statistical difference when  $P < 0.05$ . From birth to day 60 of age, heart rate had a decreasing variation, from 141 bpm to 104 bpm; the respiratory rate ranged from 59 to 53 rpm. Rectal temperature remained stable with values between 38.5 and 38.9°C. The observed data through the established physiological biomarkers of calves kept in tropical, edaphoclimatic conditions of the Amazon biome, are in line with those described in other studies of the Girolando calves, at Piauí, in 2014, where the heart rate (HR) obtained mean values of 101 bpm, rectal temperature (RT) was observed of 38.3°C, differing only in the respiratory rate (RR) which was 42 bpm.



The values obtained can be used as physiological parameters for the vital functions of calves raised in the mesological conditions of the Amazonian humid tropics.

**Keywords:** calf, vital functions, physiological parameters, Amazon



## CASOS NOTIFICADOS DE BRUCELOSE EM BOVINOS NO ESTADO DE SÃO PAULO, BRASIL, ENTRE 2017 E 2019

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Foi realizado um estudo de casos notificados de brucelose em bovinos no estado de São Paulo, Brasil, entre 2017 e 2019, a fim de verificar a progressão do controle da doença ao longo do tempo na mesma região, considerando o emprego de um programa de controle da doença iniciado em 2001, que inclui vacinação de bezerras e abate de animais positivos e permanece eficaz até hoje. Os dados foram coletados de médicos-veterinários particulares responsáveis por testar os animais em todas as regiões do estado e por meio de relatório obrigatório apresentado com todos os resultados à Coordenadoria de Defesa Agropecuária (CDA). O exame realizado pelos veterinários particulares em campo foi o teste do antígeno acidificado tamponado (AAT), que determina a presença de anticorpos contra *Brucella spp.* Todos os dados foram compilados mensalmente pela CDA ao longo destes 3 anos e depois comparados com os dados de um estudo de prevalência (DIAS, R.A. et al, 2016). Em 2017, foram testados 121.157 animais, com resultado de 44 reações positivas (0,04%). No



ano seguinte a ocorrência triplicou, com um total de 129 positivos em 106.899 animais testados (0,12%). Durante o ano seguinte, o número de positivos caiu para 45 em 84.403 animais testados (0,05%). O estudo de DIAS et al. resultou em uma prevalência de bovinos positivos de 2,4%, considerando dados coletados em 2011, 10 anos após a vacinação obrigatória de todas as bezerras com a cepa B19. O aumento do número de animais com resultado positivo em 2018 deve-se à exportação de gado vivo, o que garantiu que os animais não testados anteriormente fossem examinados sob pré-condição internacional. Os resultados dos dados coletados mostraram uma importante diminuição de animais positivos desde 2011, indicando que os atuais métodos de controle da doença são um meio eficaz para diminuir a prevalência da brucelose nos rebanhos brasileiros.

**Palavras-chave:** Brucelose, bovinos, Brasil, notificado





## NOTIFIED CASES OF BRUCELLOSIS IN BOVINES IN THE STATE OF SÃO PAULO, BRAZIL, BETWEEN 2017 AND 2019

A study of notified cases of brucellosis in bovines was conducted in the state of São Paulo, Brazil, between 2017 and 2019 in order to verify the disease control progression over time in the same region, considering the employment of a disease control program in 2001, which includes vaccination of heifers and culling of positive animals and stays effective to this day. The data was collected from private veterinarians responsible for testing the animals from all regions within the state and through mandatory report of all results to the Coordenadoria de Defesa Agropecuária (CDA). The exam performed by the private veterinarians in the field was the rose bengal test, which measures the presence of antibodies against *Brucella spp.* All data were compiled by the Veterinary Authorities on a monthly basis over these 3 years, and then compared to the data of a prevalence study (DIAS, R.A. et al, 2016). In 2017, 121,157 animals were tested, with the result of 44 positive reactions (0.04%). In the next year the occurrence tripled, with a total of 129 positives in 106,899 animals tested (0.12%). During the following year the number of positives retracted to 45 in 84,403 animals tested (0.05%). The study from DIAS et al resulted in a prevalence of positive bovines of 2.4%, considering data collected in 2011, 10 years after the mandatory vaccination of all heifers with the B19 strain. The increase in the number of animals reported positive in 2018 is due to the exportation of live cattle, which ensured that animals not previously tested were examined under international



precondition. The results from the data collected showed an important decrease in positive animals since 2011, indicating that the current methods of control of the disease are an effective means to diminish the prevalence of brucellosis in Brazilian herds.

**Keywords:** Brucellosis, bovines, Brazil, notified



## COMPARAÇÃO ENTRE AMOSTRAS GENITAIS (MUCO CERVICOVAGINAL E FRAGMENTO UTERINO) E URINA PARA DIAGNÓSTICO DA LEPTOSPIROSE BOVINA

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A leptospirose genital bovina (BGL) é uma doença crônica que causa distúrbios reprodutivos, como morte embrionária, repetição de estro, abortamentos e infertilidade. Por muitos anos, a infecção genital foi considerada um efeito secundário da infecção renal. No entanto, a BGL foi descrita como uma síndrome específica dissociada da doença renal e sistêmica. Portanto, o objetivo deste estudo foi analisar amostras genitais como muco cervical-vaginal (MCV) e fragmento uterino (FU) de vacas vivas subférteis, para diagnóstico da leptospirose bovina. Os procedimentos foram aprovados pelo Comitê de Ética para Uso Animal da Universidade Federal Fluminense (protocolo 9527220222). Amostras de urina, MCV e FU foram coletadas de 75 vacas pertencentes de quatro rebanhos do estado do Rio de Janeiro. Os rebanhos foram selecionados através de triagem sorológica através do Teste de Aglutinação Microscópica (MAT) em uma amostragem de 30 animais. Nos rebanhos com alta sororeatividade (>20% dos animais) contra o sorogrupo Sejroe (estirpes adaptadas a bovinos), realizou-se análise molecular individual de vacas com



histórico de falhas reprodutivas. Foram selecionadas 75 fêmeas negativas para *Brucella* sp., destinadas ao descarte devido a subfertilidade. As amostras de urina foram obtidas por sondagem uretral, enquanto MVC foi coletada por escova citológica na fórnix vaginal. A biópsia uterina foi realizada com pinça de Yeoman, através da via transcervical. A análise molecular foi executada através da PCR do gene *lipL32*, exclusivo de leptospiros patogênicos. Dos 75 animais coletados, 44 (58,3%) foram positivos na *lipL32*-PCR. Destes, apenas 11 (25%) tiveram amostra de urina positiva, enquanto 35 animais (79,5%) foram positivos em amostras genitais (MCV e FU). Este estudo demonstrou a associação do diagnóstico sorológico e molecular, para avaliação em nível de rebanho e individual, respectivamente. Tal protocolo se mostrou viável para o diagnóstico de animais carreadores, pois 58,3% dos animais avaliados foram positivos. Os resultados reforçam que o uso de amostras genitais é crucial para o diagnóstico de vacas carreadoras de *Leptospira* spp. Caso apenas amostras de urina fossem avaliadas, apenas 11 das 44 vacas infectadas seriam diagnosticadas. Portanto, a infecção por leptospiros em bovinos com subfertilidade é claramente subdiagnosticada quando apenas amostras de urina são avaliadas.

**Palavras -chave:** *Leptospira* spp., Reprodução, Infertilidade, Diagnóstico



## COMPARISON OF GENITAL (CERVICOVAGINAL MUCUS AND UTERINE FRAGMENT) AND URINE SAMPLES TO DIAGNOSE BOVINE LEPTOSPIROSIS

Bovine genital leptospirosis (BGL) is a chronic disease that causes reproductive disorders such as embryonic death, repeated estrus, abortion, and infertility. For many years, genital tract infection has been considered a secondary effect of renal infection. However, BGL has been recently described as a specific syndrome dissociated from renal and systemic disease. Therefore, this study aimed to analyze genital samples such as cervical-vaginal mucus (CVM) and uterine fragments (UF) from live subfertile cows to the diagnosis of BGL. Procedures were approved by the Ethical Committee for Animal Use of Federal Fluminense University (protocol 9527220222). Urine, CVM, and UF samples were collected from 75 cows from four herds in Rio de Janeiro state. Herds were selected through serologic screening using the Microscopic Agglutination Test (MAT) in a sampling of 30 animals. In the herds with high seroreactivity (>20% of the animals) against Sejroe serogroup (strains adapted to cattle), molecular analyses were performed in samples from individual cows with a history of reproductive failures. From those, 75 cows were selected, which were negative for *Brucella* sp. and culled. Urine samples were obtained with a urethral probe, while CVM samples were collected with a cytologic brush in the vaginal fornix. Uterine biopsy was performed with a Yeoman clamp through the transcervical route. The molecular analysis was carried out with PCR of the *lipL32* gene, which is exclusive of pathogenic leptospires. Of the 75 evaluated animals, 44 (58.3%)



were positive for *lipL32*-PCR. Of those, only 11 (25%) had positive in urine samples, while 35 animals (79.5%) were positive in genital samples (CVM and UF). This study demonstrated the association of serologic and molecular diagnosis, to the evaluation of herd and individual levels, respectively. This protocol was viable for the diagnosis of carriers since 58.3% of the evaluated animals were positive. The results here reinforce genital samples as a crucial point for diagnosing *Leptospira* spp. carrier cows. Only 11 of 44 infected cows would be diagnosed if just urine samples were evaluated. . Therefore, the infection by leptospires in subfertile bovines is clearly underdiagnosed when only urine samples are evaluated.

**Keywords:** *Leptospira* spp., Reproduction, Infertility, Diagnosis



## COMPARAÇÃO ENTRE O *CALIFORNIA MASTITIS TEST* E A ANÁLISE MICROBIOLÓGICA DO LEITE COMO MÉTODOS AUXILIARES PARA O DIAGNÓSTICO DA MASTITE BOVINA

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A mastite é um grande gargalo sanitário na pecuária leiteira, devido as suas implicações diretas ou indiretas, que impactam negativamente desde o produtor, indústria, até o consumidor final. Em vista disso, priorizam-se investigações acerca de contagem de células somáticas, cultura bacteriológica e tratamentos antimicrobianos. Há diversas formas de diagnosticar a mastite bovina e o exame microbiológico do leite é o método mais confiável. Um deles é o *California Mastitis Test* (CMT), este teste é considerado subjetivo e a sua interpretação pode colocar em risco programas de controle pela possibilidade de se identificar um número considerável de animais ou quartos mamários infectados como normais. Nesse sentido, o objetivo deste trabalho foi comparar o exame microbiológico do leite com o CMT como testes diagnósticos auxiliares para a mastite. Foram avaliadas 83 fêmeas bovinas primíparas e pluríparas, da raça Holandesa, em diferentes estágios de lactação, não submetidas a tratamento medicamentoso nos 30 dias anteriores à coleta de material. Os animais



pertenciam a um plantel homogêneo criado em sistema *Free Stall*, em uma propriedade comercial localizada no sudoeste do Paraná. A ordenha mecânica era realizada três vezes ao dia. Após o exame físico da glândula mamária, a secreção láctea de cada teto foi submetida ao teste de CMT e na sequência, após a higienização e antissepsia do úbere, foram colhidas amostras de cada quarto mamário para o exame microbiológico. No Laboratório de Microbiologia da Universidade Federal da Fronteira Sul, as amostras foram cultivadas em ágar sangue de ovelha (5%) com incubação em aerobiose a 37° C, com leituras de 24h. A análise estatística foi realizada pelo software Graphpad Prism 9.0® e os resultados avaliados pelo teste de Wilcoxon. A frequência de quartos negativos no CMT (n=267; 80,42%) foi maior estatisticamente do que a frequência de quartos sem isolamento de microrganismos (n=165; 49,69%). Enquanto a frequência de quartos positivos no CMT (n=98; 29,5%) foi estatisticamente menor do que a frequência de quartos positivos no exame microbiológico (n=167; 50,30%). Assim, é possível inferir que o teste de CMT pode ser utilizado para triagem de mastite em bovinos, contudo não como um teste confirmatório.

**Palavras-chave:** Glândula Mamária; Leite; Sanidade





## **COMPARISON BETWEEN CALIFORNIA MASTITIS TEST AND MICROBIOLOGICAL ANALYSIS OF MILK AS AUXILIARY METHODS FOR DIAGNOSIS OF BOVINE MASTITIS**

Mastitis is a major health problem in dairy farming, due to its direct and indirect implications, which negatively impact the producer, the industry, and even the final consumer. In view of this, investigations about somatic cell counts, bacteriological culture, and antimicrobial treatments are prioritized. There are several ways to diagnose bovine mastitis and microbiological examination of milk is the most reliable method. One of them is the California Mastitis Test (CMT), but this test is considered subjective and its interpretation can put control programs at risk because of the possibility of identifying a considerable number of infected animals or breast quarters as normal. The objective of this study was to compare the microbiological examination of milk and the CMT as auxiliary diagnostic tests for mastitis. We evaluated 83 primiparous and pluriparous Holstein females at different lactation stages that had not undergone any medication treatment in the 30 days prior to the collection of material. The animals belonged to a homogeneous herd raised in free stall system, in a commercial property located in the southwest of Paraná. Mechanical milking was performed three times a day. After physical examination of the mammary gland, milk secretion from each teat was tested for CMT and after udder hygiene and antisepsis, samples from each udder quarter were collected for microbiological examination. In the Microbiology Laboratory of the Universidade Federal da



Fronteira Sul, samples were grown on sheep blood agar (5%) with incubation in aerobiosis at 37° C, with 24h readings. Statistical analysis was performed using Graphpad Prism 9.0® software and the results were evaluated using the Wilcoxon test. The frequency of negative CMT rooms (n=267; 80.42%) was statistically higher than the frequency of rooms without isolation of microorganisms (n=165; 49.69%). While the frequency of CMT positive rooms (n=98; 29.5%) was statistically lower than the frequency of microbiological examination positive rooms (n=167; 50.30%). Thus, it can be inferred that the CMT test can be used to screen for mastitis in cattle, however not as a confirmatory test.

**Keywords:** Health; Mammary Gland; Milk



## CONDUÇÃO DIAGNÓSTICA DE TIMPANISMO ABOMASAL EM BEZERRA DA RAÇA JERSEY LACTENTE

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O timpanismo abomasal em bezerros leiteiros lactentes tem sido frequentemente relatado, devido às novas mudanças e recomendações dietéticas, que tende ao aleitamento intensivo, além do envolvimento de outros fatores de risco e agentes infecciosos. O objetivo deste resumo é descrever os desafios e condução do diagnóstico de timpanismo abomasal em uma bezerra no pré-desaleitamento, que incluiu a realização de uma série de exames complementares. Bezerra, Jersey, 32 dias de idade, colostrada (Brix = 8,4%) foi encaminhada à Clínica de Bovinos e Pequenos Ruminantes (HOVET, FMVZ/USP) com suspeita de “ruminal drinking”. Na anamnese foi reportada diarreia há 7 dias, desidratação, apatia e anorexia (ausência do reflexo de sucção), com resposta à terapia sintomática e interesse em mamar na mãe, porém manifestando timpanismo recorrente com desconforto abdominal. Exame físico: bezerra alerta, desidratada, mucosas avermelhadas, T°C retal 38,9 e fezes pastosas à líquida.



Foi detectado aumento de volume abdominal no formato pêra-maçã nos lados direito e esquerdo, detectando-se som timpânico à percussão, eventual timbre metálico, e exacerbação de crepitação na auscultação. Foi realizada a sondagem orogástrica com refluxo de 800 mL de líquido pútrido com odor ácido, coloração branco-acizentada e pH 3,2. Exames laboratoriais: hematócrito 37%, proteína sérica 4,86g/dL, fibrinogênio 1000mg/dL, neutrofilia (6.876uL segmentado/mm<sup>3</sup>), pH 7,50, pCO<sub>2</sub> 60,1 mmHg, HCO<sub>3</sub><sup>-</sup> 46,2 mEq/L, SO<sub>2</sub> 87,5%, Base excess 19,3, Ânion Gap 2,1mmol/L, sódio 124,1mEq/L, potássio 1,42mEq/L, cloreto 77,2mEq/L e cálcio iônico 0,948mmol/L, os quais revelaram alcalose metabólica com compensação respiratória e hiponatremia, hipocalcemia e hipocloremia. Foi realizada ultrassonografia abdominal e raio x contrastado, porém para a identificação e avaliação do abomaso em bezerras no pré-desaleitamento, especialmente manifestando timpanismo, requer padronização. O diagnóstico de timpanismo abomasal nesse caso foi definido com base no exame físico, e especialmente pela hemogasometria e hipocloremia, muitas vezes inacessíveis nas fazendas. Este caso foi desafiador e demonstra a demanda por pesquisas futuras avaliando a fisiopatogenia e diagnóstico das afecções que acometem o abomaso em bezerros lactentes.

**Palavras-chave:** abomaso, exames complementares, nutrição, manejo



## DIAGNOSTIC CONDUCTION OF ABOMASAL BLOAT IN SUCKLING JERSEY CALF

Abomasal bloat in suckling dairy calves has been frequently reported due to the new dietary changes and recommendations, which tend intensive milk feeding, in addition to the involvement of other risk factors and infectious agents. The objective of this abstract is to describe the challenges of the abomasal bloat diagnosis in a pre-weaning heifer calf, which included the performance of a series of ancillary tests. A 32-day old Jersey heifer calf, with history of being fed colostrum (Brix = 8.4%) was referred to the Cattle and Small Ruminants Clinic (HOVET, FMVZ/USP) with suspect of “ruminal drinking”. In the anamnesis, diarrhea for 7 days, dehydration, apathy, and anorexia (absence of the sucking reflex) were reported, with response to symptomatic therapy and interest in breastfeeding the mother, but manifesting recurrent bloat with abdominal discomfort. At physical examination the calf was alert, dehydrated, reddish mucous membranes, 38.9°C of rectal temperature, and pasty to liquid stools. A pear-apple shaped increase in abdominal volume was noticed on the right and left sides, with a tympanic sound on percussion, possible metallic timbre, and exacerbation of crackles on auscultation. Orogastric tube was performed and retrieval of approximately 800 mL of putrid content with acidic odor, grayish-white color and pH 3.2. Laboratory tests: hematocrit 37%, serum protein 4.86g/dL, fibrinogen 1000mg/dL, neutrophilia (6,876uL segmented/mm<sup>3</sup>), pH 7.50, pCO<sub>2</sub> 60.1 mmHg, HCO<sub>3</sub>-46.2 mEq/L, CO<sub>2</sub> 87.5%, Base excess 19.3, Anion Gap



2.1mmol/L, sodium 124.1mEq/L, potassium 1.42mEq/L, chloride 77.2mEq/L and ionic calcium 0.948mmol/L, which revealed metabolic alkalosis with respiratory compensation and hyponatremia, hypokalemia and hypochloremia. Abdominal ultrasound and contrast x-ray were performed, but for the identification and evaluation of the abomasum in pre-weaning calves, especially manifesting bloat, standardization is still needed. The diagnosis of abomasal bloat in this case was concluded based on physical examination, and especially on blood gas analysis and hypochloremia, which are often unavailable on farms. This case was challenging and demonstrates the demand for future research evaluating the pathophysiology and diagnosis of conditions that affect the abomasum in suckling calves.

**Keywords:** abomasum, ancillary exams, nutrition, management



## CONFORTO TÉRMICO NOS SISTEMAS DE *Compost Barn*, *Free Stall* E CRIAÇÃO A PASTO RELACIONADOS À OBSERVAÇÃO DA FREQUÊNCIA DE ALIMENTAÇÃO E TEMPO EM ESTAÇÃO

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No Brasil em regiões de clima tropical, as temperaturas durante o dia podem chegar até 40° C no período que o sol se encontra *a pino*. Dentro da bovinocultura leiteira, principalmente quando se utiliza raças europeias, a temperatura ambiental é um ponto-chave para a produção animal eficiente. O estresse térmico ocorre quando a taxa de calor corporal do animal excede a taxa de conforto térmico, prejudicando diretamente a produção, sanidade e bem-estar. Diante disto, o estudo objetivou determinar qual o horário de maior conforto térmico para ingestão de matéria seca nos sistemas *Compost Barn*, *Free Stall* e criação a pasto, bem como estabelecer qual destes fornece melhores condições de conforto térmico analisando-se dados de permanência em estação. Esse estudo foi realizado em nove (três de cada sistema) propriedades do sudoeste do Paraná, nos meses de fevereiro a abril de 2020. Foram selecionadas cinco fêmeas da raça Holandesa por propriedade, com escore corporal 2,5 a 3,0, multíparas e hígdas. Os dados de tempo de ingestão de matéria seca e tempo em estação foram coletados com auxílio de um binóculo 20 x 50 (Jumelles®). A aferição dos parâmetros foi realizada a cada dez minutos com início às 06:00 e



término às 20:00 horas. Os dados foram analisados pelo software estatístico Genes<sup>®</sup> considerando  $P > 0,05$ . Nos três sistemas a frequência de alimentação foi adequada, tendo em vista que o tempo de alimentação fisiológico para bovinos é de 8 horas distribuídas ao longo do dia. O maior consumo de matéria seca foi durante a manhã (6:00 às 10:00hrs) provando que os animais se alimentam com maior frequência em temperaturas amenas. Pesquisadores relatam que os bovinos permanecem em média 30,06% do seu tempo durante o dia em estação, valores superiores a isso podem demonstrar quadros de estresse térmico. Em sistema *Compost Barn* os animais apresentaram média de 37,44% de permanência do tempo em estação, enquanto no sistema *Free Stall* a média foi de 48,3%. Já no sistema a pasto, os bovinos permaneceram 56,25% do tempo em pé realizando trocas de calor, indicando que estes dois sistemas não oferecem condições adequadas para manutenção da homeostasia térmica.

**Palavras-chave:** Ambiente. Bem-estar. Comportamento. Estresse





## **HEAT COMFORT IN *Compost Barn*, *Free Stall* AND PASTURE RAISING SYSTEMS RELATED TO FEEDING FREQUENCY AND STATION PERMANENCE TIME OBSERVATION**

In Brazil, in regions with tropical climates, daytime temperatures can reach up to 40° C when the sun is at its peak. In dairy cattle farming, especially when using European breeds, environmental temperature is a key point for efficient animal production. Heat stress occurs when the animal's body heat rate exceeds the thermal comfort rate, directly harming production, health, and well-being. Therefore, the study aimed to determine which is the most thermally comfortable time for dry matter ingestion in *Compost Barn*, *Free Stall* and pasture rearing systems, as well as to establish which one provides better thermal comfort conditions by analyzing station permanence data. This study was conducted in nine (three of each system) farms in the southwest of Paraná, from February to April 2020. Five females of the Holsteins breed were selected per property, with body score 2.5 to 3.0, multiparous and healthy. Data on dry matter intake time and time in station were collected with the aid of a 20 x 50 binocular (Jumelles®). The parameters were measured every ten minutes starting at 06:00 and ending at 20:00. The data were analyzed by statistical software Genes® considering  $P > 0.05$ . In all three systems the feeding frequency was adequate, considering that the physiological feeding time for cattle is 8 hours distributed throughout the day. The highest dry matter consumption was during the morning (6:00 am to 10:00 am) proving that the animals feed more frequently in mild temperatures.



Researchers report that cattle remain on average 30.06% of their time during the day in station, values higher than this may demonstrate heat stress. In the *Compost Barn* system the animals presented an average of 37.44% of time spent in the station, while in the *Free Stall* system the average was 48.3%. In the pasture system, the bovines remained 56.25% of the time standing and exchanging heat, indicating that these two systems do not offer adequate conditions for maintaining thermal homeostasis.

**Keywords:** Behavior. Environment. Stress. Welfare



## DESCRIÇÃO DE SETE CASOS DE DISTOCIAS EM FÊMEAS BOVINAS

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A distocia pode ser definida como dificuldade de parição resultante de parto espontâneo prolongado ou extração assistida prolongada ou grave. . Pode ter origem materna ou fetal e a incidência total de casos em bovinos pode ultrapassar 10%. Resulta em riscos para a vida da parturiente e do neonato. O objetivo deste resumo é descrever casos de distocia atendidos na Clínica de Bovinos e Pequenos Ruminantes da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (FMVZ/USP) entre 2012 e 2022. Neste período foram atendidos 7 bovinos, 4 caprinos, 8 ovinos e 4 suínos, totalizando 23 fêmeas com dificuldade de parto. Destes, observou-se 26% de casos de distocia em mini animais, sendo 4 ocorrências em mini porcos, 1 em mini cabra e 1 em mini vaca. No estudo em questão, serão descritos os casos nos bovinos. A maioria dos animais chegou para atendimento clínico com mais de 12 horas de início do trabalho de parto, com grau de desidratação leve a moderado, em decúbito e realizou-se 3 cesarianas e 4 procedimentos de tração forçada. A média das frequências cardíacas foi de 100 bpm, da frequência respiratória de 65 mpm e temperatura de 37,7°C, sinalizando quadros de dor. As mucosas se



apresentavam congestas na maioria dos casos, a taxa de sobrevivência fetal foi de 0% e a de sobrevivência materna foi de 71,4%. Em todos os casos clínicos, a causa da distocia nas fêmeas bovinas foi de origem fetal: mau posicionamento fetal, desproporção feto-pélvica e morte fetal, com maceração fetal em um dos casos clínicos. Com base neste levantamento concluiu-se que o grande intervalo de tempo entre o início do trabalho de parto, juntamente com a observação de dificuldade por parte da parturiente em expulsar o produto, e o encaminhamento para o atendimento obstétrico, culminou nas mortes fetais e, em alguns casos, na morte materna. Além disso, deduziu-se que os animais, em sua maioria, não possuem o devido manejo pré-parto e que também não há o planejamento dos cruzamentos a fim de evitar a gestação de produtos muito grandes e que são incompatíveis com o canal cervical.

**Palavras-chave:** Parto, Bezerro, Intervenção



## DESCRIPTION OF SEVEN CASES OF DYSTOCIA IN BOVINE FEMALES

Dystocia can be defined as difficulty in delivery resulting from prolonged spontaneous delivery or prolonged or severe assisted extraction. . It can be of maternal or fetal origin and the total incidence of cases in cattle can exceed 10%. It results in risks to the life of the parturient and the neonate. The objective of this summary is to describe dystocia cases treated at the Cattle and Small Ruminants Clinic of the Faculty of Veterinary Medicine and Animal Science at the University of São Paulo (FMVZ/USP) between 2012 and 2022. During this period, 7 cattle, 4 goats, 8 sheep and 4 pigs, totaling 23 females with birth difficulties. Of these, 26% of dystocia cases were observed in mini animals, with 4 occurrences in mini pigs, 1 in mini goat and 1 in mini cow. In the study in question, cases in cattle will be described. Most animals arrived for clinical care more than 12 hours after the onset of labor, with a mild to moderate degree of dehydration, in recumbency, and 3 cesarean sections and 4 forced traction procedures were performed. The mean heart rate was 100 bpm, respiratory rate was 65 mpm and temperature was 37.7°C, signaling pain. The mucous membranes were congested in most cases, the fetal survival rate was 0% and the maternal survival rate was 71.4%. In all clinical cases, the cause of dystocia in bovine females was of fetal origin: fetal malposition, fetal-pelvic disproportion and fetal death, with fetal maceration in one of the clinical cases. Based on this survey, it was concluded that the long time interval between the beginning of labor, together with the observation of



difficulty on the part of the parturient in expelling the product, and the referral to obstetric care, culminated in fetal deaths and, in some cases, in maternal death. In addition, it was deduced that the animals, for the most part, do not have the proper prepartum management and that there is also no planning of crosses in order to avoid the gestation of very large products that are incompatible with the cervical canal.

**Keywords:** Childbirth, Calf, Intervention



## DETECÇÃO DE *MANNHEIMIA HAEMOLYTICA* EM BIOFILME DENTAL DE BEZERROS

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As doenças periodontais são distúrbios que afetam o desempenho e o bem-estar animal em ruminantes e são causadas primariamente pela disbiose do biofilme bucal e diversos fatores extrínsecos ao hospedeiro. Estudos preliminares com o emprego do sequenciamento de próxima geração tem revelado que a composição da microbiota dental de bezerros é bastante complexa. Assim este estudo teve como objetivo caracterizar a microbiota dental de bezerros criados extensivamente e com diferentes condições clínicas periodontais utilizando-se da técnica de sequenciamento de alto rendimento do gene 16s rRNA. Os animais foram classificados em três grupos: sítios periodontais saudáveis (HPS), sítios periodontais com gengivite (GPS) e sítios periodontais com supuração (SPS) a partir de exame clínico-bucal dos incisivos e as amostras foram coletadas diretamente do biofilme dental subgengival. O sequenciamento resultou em 291 ASVs (amplicon sequence variance), as quais 41 ASVs foram compartilhadas entre os grupos HPS, GPS e SPS. *Mannheimia haemolytica*, é um patógeno-chave que está frequentemente associado à microbiota comensal nasofaríngea



de bovinos e envolvido em doenças respiratórias nestes animais, foi detectada em abundância variável na microbiota dental de todos os três grupos. Na saúde bucal, o papel desse microrganismo e suas interações com a microbiota local é pouco conhecido, principalmente em ruminantes. Apesar da complexidade, da riqueza e diversidade da microbiota presente no ecossistema bucal, os resultados do presente estudo indicam que a composição qualitativa da microbiota subgengival de bovinos com diferentes condições clínicas, tem particularidades específicas, com semelhanças e diferenças entre si, o que inclui possíveis correlações com o microbioma do ecossistema respiratório.

**Palavras-chave:** Bezerros, doença periodontal, microbiota





## DETECTION OF *MANNHEIMIA HAEMOLYTICA* IN DENTAL BIOFILM OF CALVES

Periodontal diseases are disorders that affect performance and animal welfare in ruminants and are primarily caused by oral biofilm dysbiosis and several factors extrinsic to the host. Preliminary studies using next-generation sequencing have revealed that the composition of the calf dental microbiota is quite complex. Thus, this study aimed to characterize the dental microbiota of calves raised extensively and with different periodontal clinical conditions using the technique of high throughput sequencing of the 16s rRNA gene. The animals were classified into three groups: healthy periodontal sites (HPS), periodontal sites with gingivitis (GPS) and periodontal sites with suppuration (SPS) from clinical-oral examination of the incisors and samples were collected directly from the subgingival dental biofilm. The sequencing resulted in 291 ASVs (amplicon sequence variance), of which 41 ASVs were shared between the HPS, GPS and SPS groups. *Mannheimia haemolytica*, a key pathogen that is frequently associated with the commensal nasopharyngeal microbiota of cattle and involved in respiratory diseases in these animals, was detected in variable abundance in the dental microbiota of all three groups. In oral health, the role of this microorganism and its interactions with the local microbiota is poorly understood, especially in ruminants. Despite the complexity, richness and diversity of the microbiota present in the oral ecosystem, the results of the present study indicate that the qualitative composition of the subgingival microbiota of cattle with different clinical



conditions has specific particularities, with similarities and differences between them, which includes possible correlations with the respiratory ecosystem microbiome.

**Keywords:** Calves, periodontal disease, microbiota



## DINÂMICA DA IgG E ANTICORPOS ESPECÍFICOS CONTRA OS ENTEROPATÓGENOS NO LEITE DE TRANSIÇÃO DE VACAS GIROLANDO

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O leite de transição é a secreção mamária obtida entre a 2<sup>a</sup> e 6<sup>a</sup> ordenha pós-parto, rico em proteínas, IgG, gordura, lactose e oligossacarídeos, e corroboram com desenvolvimento das vilosidades intestinais e saúde das bezerras. O objetivo desta pesquisa foi avaliar a dinâmica de IgG e anticorpos específicos contra os enteropatógenos na secreção mamária entre a 2<sup>a</sup> e 43<sup>a</sup> ordenha. Foram incluídas 18 fêmeas primíparas e 33 multíparas. Secreção mamária foi obtida na 2<sup>a</sup> e 9<sup>a</sup>, 11<sup>a</sup>, 13<sup>a</sup>, 15<sup>a</sup>, 29<sup>a</sup> e 43<sup>a</sup> ordenha pós-parto. A IgG total determinada entre a 2<sup>a</sup> e 43<sup>a</sup> ordenha, pelo método ELISA *sandwich*; e os anticorpos específicos contra enteropatógenos nas ordenhas 1, 2, 6 e 13, por meio de kits comerciais ELISA (Bio-X *Diagnostics* S.A.) utilizando amostras puras. Em novilhas a produção de leite de transição entre a 2<sup>a</sup> e 6<sup>a</sup> ordenha foi 4.19±0.33L, a partir da 7<sup>a</sup> ordenha a produção média em novilhas foi de 6.63±0.35, Brix 15.24%, ELISA 51.76±3.69. Já em multíparas a produção de leite de transição foi 5.71±3.29 L, após a 7<sup>a</sup> ordenha, produção média foi 9.96±0.22 L, Brix 13.69%, ELISA 47.25±2.03 apresentando diferença no tempo (P≤0.0001). Anticorpos específicos apresentou diferença no CP alpha



( $P=0.0026$ ), primíparas apresentaram maiores valores  $52.34\pm 3.49$  e multíparas  $52.17\pm 2.74$ . Os anticorpos apresentaram diferença no tempo ( $P\leq 0.0001$ ). Em novilhas o Coronavírus foi  $75.41\pm 3.39$ , Rotavírus  $66.70\pm 0.85$ , CPalpha e multíparas  $76.09\pm 2.54$ , Rotavírus  $68.06\pm 3.69$  em novilhas e em multíparas, Clostridium  $52.34\pm 3.49$ . Em multíparas o Coronavírus foi  $76.09\pm 2.54$ , Rotavírus  $66.70\pm 0.56$ , CPalpha  $52.17\pm 2.74$ , CpBeta  $76.60\pm 2.65$ , Epsilon  $84.05\pm 2.15$  e E.coli  $56.39\pm 3.72$  em multíparas. Os valores oscilaram, no entanto, houve um decréscimo nos anticorpos para primíparas. Desta forma existem evidências que os animais foram desafiados por patógenos e isso está relacionado com alterações dos valores de anticorpos específicos, comprovando que vacinação e cuidados no manejo é de suma importância para o rebanho. A paridade pode influenciar a produção de colostro, principalmente sobre a qualidade imunológica, responsável por realizar uma boa transferência de imunidade passiva. Vacas multíparas apresentam valores mais altos em relação a qualidade imunológica devido ao maior tempo exposição a patógenos, levando em consideração os anos e as vacinações

**Palavras-chave:** Imunidade, Secreção mamária, vacas mestiças, ELISA, paridade, ordenhas



## **IgG AND SPECIFIC ANTIBODIES DYNAMIC AGAINST ENTEROPATHOGENS IN TRANSITION MILK OF HOLSTEIN X GIR CROSSBRED COWS**

The Holstein x Gir crossbreed (Girolando) contributes with 80% of milk production in Brazil, due to its resistance profile and adaptation to the climatic conditions of subtropical and tropical countries. Studies specifically for the Girolando breed are scarce, and in our knowledge there are none about immune quality of the colostrum of Girolando heifers and cows. The main objective of this research was to determine the quality of colostrum of Girolando cows with history of pre-calving vaccination against neonatal diarrhea, through colostrometer, Brix refractometer, colostrum balls, besides evaluation of total IgG level and specific antibodies against enteropathogens, using ELISA sandwich and commercial ELISA competition kits (Bio-X Diagnostics S.A.). 18 primiparous and 33 multiparous Girolando females were included. Colostrum was obtained up to 2 hours postpartum. The volume of colostrum (liters) and the IgG mass calculated by multiplication volume x concentration determined by gold-standard procedure (ELISA sandwich) were recorded. Total and specific IgG against enteropathogens were measured in cow serum and in centrifuged colostrum. The total volume of colostrum produced was  $2,56 \pm 0,30$ kg, the immune quality evaluated through ELISA sandwich was  $100,2 \pm 5,32$ , colostrometer  $74,06 \pm 6,01$ mg/ml, colostrum balls  $1.051 \pm 2,64$  and Brix  $25,08 \pm 0,77\%$ . The IgG mass calculation was  $207.16 \pm 23.14$ . Primiparous produced  $2.75 \pm 0.76$  kg of colostrum, ELISA  $98.12 \pm 9.44$ , colostrometer  $61.42 \pm 10.57$ , Brix  $23.29 \pm 1.69\%$ , colostrum balls  $1.045 \pm 4,62$  and IgG mass value of  $207,16 \pm 17,55$ . In multiparous, ELISA was  $101,46 \pm 6,51$ , colostrometer  $79.96 \pm 7,18$ mg/mL, Brix  $26,03 \pm 0.77\%$ , colostrum balls  $1.054 \pm 3.14$  and IgG mass was  $274.74 \pm 17.90$ . Specific antibodies against *Coronavirus*, *Rotavirus*, *Clostridium* (CP Alpha, Beta and Epsilon) and



*E. coli* showed differences over time ( $P \leq 0.0001$ ) presenting variation between moments of studies, some pathogens increased over the study, such as CP beta and epsilon. The animals were challenged at some point in the postpartum period, decreasing immunity consequently increasing specific antibodies. More studies should be carried out comparing crossbred with Holstein cows.

**Keywords:** Immunity, mammary secretion, crossbred cows, ELISA, number of calvings, milking



## **EFEITO DA DIARREIA EM BEZERROS LEITEIROS NA MICROBIOTA INTESTINAL DURANTE O PRIMEIRO MÊS DE VIDA**

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Este estudo teve como objetivo avaliar a influência do complexo diarreico na microbiota intestinal durante o período neonatal de bezerros. Vinte e seis bezerros leiteiros foram triados imediatamente após o nascimento e monitorados nos tempos: D13-15; D20-23 e D27-30 dias de vida. As amostras fecais foram coletadas diretamente da ampola retal usando luvas estéreis. A presença de diarreia foi mensurada pela avaliação da matéria seca (MS) das fezes e, com isso, os bezerros foram distribuídos em dois grupos: com (MS <15%) e sem (MS >15%) diarreia. Para avaliar as populações de bactérias indicadoras nas fezes, a extração de DNA foi realizada pela primeira vez usando um kit comercial. A quantificação do material genético de *Bifidobacterium* spp., *Lactobacillus* spp., *C. perfringens*, *Escherichia coli* e DNA de bactérias totais (16S rRNA) foi realizada por PCR em tempo real. A frequência de diarreia apresentada pelos bezerros foi de 73,08%, 23,08% e 26,92% em D13-15, D20-23 e D27-28, respectivamente. Os bezerros com diarreia apresentaram menor número de cópias da bactéria pesquisada, quando comparados ao grupo que não teve diarreia, principalmente em D13-15 e D27-30. O perfil apresentado pelos animais



em relação ao total de bactérias mostrou diferença significativa no D13-15, e tendência no D27-30. Animais com diarreia apresentaram menos cópias de bactérias totais (16S rRNA), quando comparados a animais com fezes normais. A população de *Bifidobacterium* spp. foi influenciada pela presença de diarreia no D13-15, quando houve diferença significativa com valores menores para o número de cópias para o grupo com diarreia em relação ao grupo com fezes normais. *Lactobacillus* spp. apresentou perfil semelhante ao de *Bifidobacterium* spp. em D13-15. Em relação à *Escherichia coli*, foi detectada diferença entre os grupos no D20-23. *C. perfringens* foi diferente apenas no D27-30, com menores valores de número de cópias para animais com diarreia. Concluiu-se que a presença de diarreia em bezerros influenciou a população de bactérias intestinais relacionadas à saúde intestinal. Além disso, a população de bactérias com potencial patogênico reduziu o número de bactérias indicadoras investigadas nas fezes dos bezerros.

**Palavras-chave:** gado leiteiro; *Escherichia coli*; colonização intestinal; *Lactobacillus* spp.; período neonatal





## EFFECT OF DIARRHEA IN DAIRY CALVES ON GUT MICROBIOTA DURING THE FIRST MONTH OF LIFE

This study aimed to evaluate the influence of diarrhea complex in the gut microbiota during the neonatal period of calves. Twenty-six dairy calves were screened immediately after birth and monitored during the times: D13-15; D20-23, and D27-30 days of life. Fecal samples were collected directly from the rectal ampoule using sterile gloves. The presence of diarrhea was measured by evaluating the dry matter (DM) of the feces and, with that, the calves were distributed into two groups: with (DM <15%) and without (DM >15%) diarrhea. To assess the populations of indicator bacteria in feces, DNA extraction was first performed using a commercial kit. The quantification of the genetic material of *Bifidobacterium* spp., *Lactobacillus* spp., *C. perfringens*, *Escherichia coli* and DNA of total bacteria (16S rRNA) was performed using real-time PCR. The frequency of diarrhea presented by calves was 73.08%, 23.08% and 26.92% in D13-15, D20-23 and D27-28, respectively. Calves with diarrhea had lower number of copies of the surveyed bacteria, when compared to the group that did not have diarrhea, especially in D13-15 and D27-30. The profile presented by the animals in relation to the total bacteria showed a significant difference in D13-15, and a trend in D27-30. Animals with diarrhea had fewer copies of total bacteria (16S rRNA), when compared to animals with feces normal. The population of *Bifidobacterium* spp. was influenced by the presence of diarrhea in D13-15, when there was a significant difference with lower values for the number of copies for the group with diarrhea compared to normal feces group. *Lactobacillus* spp. had



a similar profile to *Bifidobacterium* spp. at D13-15. Regarding *Escherichia coli*, a difference was detected between groups at D20-23. *C. perfringens* was different only at D27-30, with lower values of number of copies for animals with diarrhea. It was concluded that the presence of diarrhea in calves influenced the population of intestinal bacteria related to intestinal health. Besides that, the population of bacteria with pathogenic potential reduced the number of the investigated bacteria indicators in the calves' feces.

**Keywords:** dairy cattle; *Escherichia coli*; gut colonization; *Lactobacillus* spp.; neonatal period



## ENDOCARDITE VALVULAR EM UMA BEZERRA- RELATO DE CASO

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Foi atendida no Hospital Veterinário da Universidade Federal de Alagoas uma bezerra Nelore de 25 dias, que há 12 dias estava triste, com dificuldade para respirar e alimentar-se e com o umbigo infeccionado. No exame físico observou-se decúbito esternal, magreza, apatia, desidratação de 10%, taquicardia, taquipneia, secreção periocular, mucosa oral hipocorada, temperatura retal de 38,5°C, narinas ressecadas, respiração ruidosa, dispneia expiratória e, na ausculta pulmonar, crepitação bilateral. Umbigo aumentado e sensível e, na palpação profunda, havia aumento direcionado cranialmente, sugerindo onfaloflebite. Para tratar o quadro respiratório utilizou-se Flunixinina Meglumina e Florfenicol, além de reposição hidroeletrólítica e aporte nutricional. Os sinais progrediram, observando-se emagrecimento progressivo, apatia, dificuldade de alimentar-se, fezes com sangue e muco, anemia intensa, sopro cardíaco, estase venosa positiva, jugular ingurgitada, edema de barbela e vasos episclerais ingurgitados, diante disso, suspeitou-se de endocardite. Alterou-se o antibiótico para amoxicilina tri-hidratada durante cinco dias. Na avaliação ultrassonográfica do coração havia espessamento importante da válvula tricúspide, sugerindo a presença de neoformação vegetante na estrutura, além de líquido livre no



pericárdio. Ao Doppler visualizou-se regurgitação com abaulamento do átrio direito. O animal morreu 16 dias após entrada no hospital, sendo encaminhado à necropsia. O coto umbilical estava espessado, havia líquido peritoneal turvo na cavidade abdominal; fígado aumentado com parênquima com aspecto de noz moscada; rins escurecidos na pelve e córtex; hemorragias petequiais na bexiga, baço aumentado e intestino com hemorragias em diversos seguimentos. Edema na região de peito e barbela, líquido turvo na cavidade torácica, aumento de líquido no saco pericárdico e áreas de atalactasia nos pulmões. Na válvula tricúspide havia espessamento e proliferação de tecido granuloso de aspecto vegetativo e de coloração amarelada crescendo em direção à artéria. No histopatológico verificou-se endocardite necrosante, miocardite granulomatosa, hemorragia hepática, pneumonia intersticial, glomerulonefrite e enterite. Acredita-se que a origem da endocardite deste caso seja a onfalopatia, pois esta pode proporcionar disseminação de trombos bacterianos como consequência de bacteremias e septicemias. Apesar de não ser frequente em bezerros, a endocardite pode estar presente em rebanhos com falhas no manejo sanitário de neonatos, associada a doenças como onfalopatias e broncopneumonias, levando a óbito dos acometidos.

**Palavras-chave:** ruminante, válvula tricúspide, onfalopatias



## VALVULAR ENDOCARDITIS IN A CALF- CASE REPORT

A 25-day-old Nelore heifer was treated at the Veterinary Hospital of the Federal University of Alagoas, who had been sad for 12 days, had difficulty breathing and feeding and had an infected navel. Physical examination revealed sternal recumbency, thinness, apathy, 10% dehydration, tachycardia, tachypnea, periocular secretion, pale oral mucosa, rectal temperature of 38.5°C, dry nostrils, noisy breathing, expiratory dyspnea and, on auscultation, pulmonary, bilateral crackles. Enlarged and sensitive umbilicus and, on deep palpation, there was an increase directed cranially, suggesting omphalophlebitis. To treat the respiratory condition, Flunixin Meglumine and Florfenicol were used, in addition to hydroelectrolytic replacement and nutritional support. The signs progressed, with progressive weight loss, apathy, difficulty in eating, bloody stools and mucus, intense anemia, heart murmur, positive venous stasis, engorged jugular, dewlap edema and engorged episcleral vessels. if of endocarditis. The antibiotic was changed to amoxicillin trihydrate for five days. In the ultrasound evaluation of the heart, there was significant thickening of the tricuspid valve, suggesting the presence of vegetative neoformation in the structure, in addition to free fluid in the pericardium. Doppler showed regurgitation with bulging of the right atrium. The animal died 16 days after admission to the hospital, being referred for necropsy. The umbilical stump was thickened, there was cloudy peritoneal fluid in the abdominal cavity; enlarged liver with nutmeg-like parenchyma; darkened kidneys in the pelvis and cortex; petechial hemorrhages in the bladder, enlarged spleen and intestine with hemorrhages in several segments. Edema in the chest



and dewlap region, cloudy fluid in the chest cavity, increased fluid in the pericardial sac and areas of atelectasis in the lungs. In the tricuspid valve there was thickening and proliferation of granular tissue with a vegetative aspect and yellowish color growing towards the artery. Histopathological findings showed necrotizing endocarditis, granulomatous myocarditis, hepatic hemorrhage, interstitial pneumonia, glomerulonephritis and enteritis. It is believed that the origin of the endocarditis in this case is omphalopathy, as this can lead to the dissemination of bacterial thrombi as a consequence of bacteremia and septicemia. Although not frequent in calves, endocarditis can be present in herds with failures in the sanitary management of neonates, associated with diseases such as omphalopathies and bronchopneumonia, leading to the death of those affected.

**Keywords:** ruminant, tricuspid valve, omphalopathies



## EPIDEMIOLOGIA DA MASTITE NO PÓS-PARTO IMEDIATO E SEU IMPACTO NA LACTAÇÃO DE VACAS LEITEIRAS

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A mastite é caracterizada pela enfermidade que mais acomete vacas leiteiras adultas. As perdas econômicas envolvem perdas na produção de leite, medicamentos, leite descartado, assistência veterinária, descarte, mão de obra e penalidades pelo laticínio. A ocorrência de mastite no pós-parto imediato e seu impacto na produção de leite ainda são pouco estudados no Brasil, em clima tropical. Assim, os objetivos desse projeto foram: 1) descrever a distribuição de patógenos isolados no pós-parto imediato; 2) avaliar o impacto da ocorrência da mastite no pós-parto no restante da lactação. Foram utilizados seis rebanhos leiteiros. Amostras de leite foram colhidas de forma asséptica no primeiro dia pós-parto. Dados zootécnicos necessários para a análise foram coletados diretamente dos programas de gerenciamento das propriedades participantes. Foram coletados mensalmente: produção de leite, contagem de células somáticas, data do parto, eventos de saúde, como mastite e outras doenças, descarte e data de secagem. Das 6.345 amostras de leite de vacas recém-paridas analisadas, os patógenos mais prevalente foram estafilococos



coagulase-negativa (30,06%; N = 1.907), seguido por levedura (1,81%; N = 115), *Streptococcus dysgalactiae* (1,36%; N = 86), *Klebsiella* spp (1,28%; N = 81) e *Corynebacterium bovis* (1,21%; N = 77). As maiores e mais prolongadas perdas de produção de leite foram causadas por patógenos contagiosos: *Staphylococcus aureus* com 1,15% (N = 73) e *Streptococcus agalactiae* com 0,14% (N = 9), as quais variaram de 0,74 a 6,78 kg/dia, dependendo do mês da lactação. No primeiro mês de lactação, as maiores perdas foram decorrentes de infecções causadas por *Staphylococcus aureus*, patógenos refratários e corineformes. No entanto, vacas infectadas por *Corynebacterium* spp recuperaram a produção a partir da metade da lactação. Ao comparar as curvas de lactação entre animais infectados e não infectados imediatamente após o parto, notou-se uma diferença inicial na produção de leite de aproximadamente 3 kg de leite e pico de produção no quinto mês. Os resultados deste estudo enfatizam a importância da realização de cultura no pós-parto, sendo imprescindível para diagnóstico e manejo destes casos no início da lactação.

**Palavras-chave:** Qualidade do leite, sanidade bovina, saúde pública





## EPIDEMIOLOGY OF MASTITIS IN THE IMMEDIATE POSTPARTUM PERIOD AND ITS IMPACT ON LACTATION IN DAIRY COWS

Mastitis is characterized by the disease that most affects adult dairy cows. Economic losses involve losses in milk production, medicines, discarded milk, veterinary care, disposal, labor and dairy penalties. The occurrence of mastitis in the immediate postpartum period and its impact on milk production are still poorly studied in Brazil, in a tropical climate. Thus, the objectives of this project were: 1) to describe the distribution of pathogens isolated in the immediate postpartum period; 2) to assess the impact of postpartum mastitis on the remainder of lactation. Six dairy herds were used. Milk samples were collected aseptically on the first postpartum day. Zootechnical data necessary for the analysis were collected directly from the management programs of the participating properties. We collected monthly: milk production, somatic cell count, calving date, health events such as mastitis and other diseases, disposal and drying date. Of the 6,345 milk samples from newly calved cows analyzed, the most prevalent pathogens were coagulase-negative staphylococci (30.06%; N = 1,907), followed by yeast (1.81%; N = 115), *Streptococcus dysgalactiae* (1.36%; N=86), *Klebsiella* spp (1.28%; N=81) and *Corynebacterium bovis* (1.21%; N=77). The largest and most prolonged losses of milk - production were caused by contagious pathogens: *Staphylococcus aureus* with 1.15% (N = 73) and *Streptococcus agalactiae* with 0.14% (N = 9), which ranged from 0.74 to 6.78 kg/day, depending on the month of lactation. In the first month of lactation, the greatest losses were



due to infections caused by *Staphylococcus aureus*, refractory and coryneform pathogens. However, cows infected by *Corynebacterium* spp recovered production from mid-lactation. When comparing lactation curves between infected and uninfected animals immediately after parturition, an initial difference in milk production of approximately 3 kg of milk and peak production at the fifth month was noted. The results of this study emphasize the importance of performing a culture in the postpartum period, which is essential for the diagnosis and management of these cases at the beginning of lactation.

**Keywords:** Milk quality; Bovine health; Public health



## FRIBROPAPILOMA GENITAL EM GARROTES: RELATO DE CASO

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Fibropapiloma genital é um tumor auto limitante de indução viral causado pelo *Herpes vírus* bovino tipo 1. Em machos, atinge pênis e prepúcio, e histologicamente é caracterizado por massas invasivas locais, com grande quantidade de tecido fibroso e envolto na superfície por células epiteliais neoplásicas, com neovascularização e diversos graus de pleomorfismo. Os sinais clínicos incluem nódulos em formato de couve flor que alteram a região genital provocando perda de libido, sangramentos, fimose, parafimose e disúria. Embora seu aspecto morfológico seja característico, fica evidente a importância da análise histopatológica para comprovar diagnóstico, avaliar prognóstico, bem como a possibilidade de recidiva. O objetivo deste trabalho foi relatar dois casos de fibropapiloma atendidos pela clínica escola veterinária - CEVET, da UNICENTRO, em garrotes não castrado, de 11 meses, da raça angus e com peso aproximado de 350kg. O primeiro animal apresentava um nódulo de aproximadamente 6 cm no óstio prepucial, outro de 4 cm a em corpo do pênis, causando parafimose. Ainda existiam múltiplos nódulos pequenos em corpo do pênis e mucosa interna do prepúcio. O segundo caso apresentava um nódulo em glândula peniana de aproximadamente 5 cm de diâmetro, causando estenose uretral e sangramento durante a micção e mais 2 nódulos pequenos, sendo um



no corpo do pênis e outro em mucosa prepucial interna. Ambos os animais foram submetidos a extirpação cirúrgica das massas maiores após previa sedação com xilazina 0,2 mg/kg e anestesia local com lidocaína. Os animais foram acompanhados durante 105 dias, não sendo encontrada recidiva no primeiro animal, no qual os tumores não removidos desapareceram espontaneamente. Houve recidiva da massa no segundo animal 30 dias após a cirurgia, com cerca de 1,5 cm, a qual foi removida por criocirurgia, e após 30 dias, houve retorno dos sinais novamente. O exame histopatológico demonstrou que a massa do segundo animal apresentava células epiteliais com intenso pleomorfismo celular associado a intensa neovascularização e fibroplasia difusa, responsável por maior capacidade infiltrativa e quando não removido com ampla margem de segurança, facilita o processo de recidiva, permitindo concluir que a análise histopatológica é a chave para estabelecer as condutas cirúrgicas mais adequadas.

**Palavras-chave:** *Herpes vírus* bovino, prepúcio, Pênis, pleomorfismo



## STEERS GENITAL FIBROPAPILLOMA: REPORT CASE

Genital fibropapilloma is a self-limiting viral-inducing tumor caused by bovine herpes virus type 1. In bulls, it affects the penis and prepuce. Histologically is characterized by local invasive masses, with a large amount of fibrous tissue and surrounded by neoplastic epithelial cells, with newly formed vessels and degrees of pleomorphism. Clinical signs include cauliflower-shaped nodules that alter the genital region causing loss of libido, bleeding, phimosis, paraphimosis and dysuria. Although its morphological appearance is characteristic, the importance of histopathological analysis is evident to confirm diagnosis, assess prognosis, as well as the possibility to recurrence. The objective of this study was to report two cases of steers' fibropapilloma treated at the veterinary school clinic – CEVET- UNICENTRO, in two uncastrated, 11-month-old angus with an approximate weight of 350kg. The first animal had a nodule measuring approximately 6 cm in the preputial ostium, another of 4 cm in the body of the penis, causing paraphimosis, both had relatively broad pedicle. There were still multiple small nodules in genital region. The second case had a nodule on the glans penis of approximately 5 cm in diameter, causing urethral stenosis with relatively broad pedicle and bleeding during urination, and 2 more small nodules (body of the penis and in internal preputial mucosa). Both animals underwent surgical removal of the larger masses after sedation with xylazine and local anesthesia with lidocaine. The animals were followed up for 105 days, and no recurrence was found in the first animal, and the unremoved tumors disappeared



spontaneously. There was a recurrence of the mass in the second animal, 30 days after surgery. It was measuring about 1.5 cm, and it was removed by cryosurgery, after 30 days the signs returned again. The mass of the second animal had cells with intense cellular pleomorphism associated with intense neovascularization and diffuse fibroplasia, responsible for high infiltrative capacity, and when it was not removed with a wide margin of safety, it facilitates the process of recurrence, allowing us to conclude that the histopathological analysis is the key to establishing the most appropriate surgical procedures.

**Keywords:** Bovine herpes virus, foreskin, penis, pleomorphism



## FOTOSSENSIBILIZAÇÃO HEPATÓGENA POR *Brachiaria* spp. ASSOCIADA A FASCIULOSE EM VACA NELORE: RELATO DE CASO

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A fotossensibilização hepatógena por *Brachiaria* spp. é uma intoxicação comum em ruminantes devido a deposição de saponinas esteroidais que leva à colestase e acúmulo do pigmento fotodinâmico, filoteritina na circulação periférica. A lesão hepática torna-se mais acentuada quando associada ao parasitismo por *Fasciola hepatica*, havendo perda da funcionalidade e obstruções canaliculares. Objetiva-se relatar um caso de fotossensibilização hepatógena por *Brachiaria* spp. em vaca associado a fasciolose, atendido pelo Hospital Veterinário da Universidade Federal do Espírito Santo. O animal de seis anos, Nelore, criado em regime extensivo de *Brachiaria* spp., em Jerônimo Monteiro/ES, com histórico de dermatite sem resposta ao tratamento. Clinicamente apresentava dermatite multifocal com intensa necrose e exsudação serosanguinolenta, com áreas secas e fibrosas multifocais a coalescentes com predomínio em cabeça, região: submandibular; músculo semimembranoso e semitendinoso; articulação tarso metatársica e femurotibiopatelar; axilares e cauda. O hemograma revelou anemia normocítica hipocrômica, hiperproteinemia



e leucocitose por neutrofilia. No perfil bioquímico evidenciou aumento dos valores de proteína total, globulinas, aspartato aminotransferase, fosfatase alcalina, creatina quinase, gamaglutamil transferase, bilirrubinas direta e indireta e ureia. Foi realizada biópsia hepática evidenciando intensa degeneração de hepatócitos multifocal, discreta hiperplasia multifocal de ductos e moderada fibrose periportal. A fasciolose foi confirmada por meio da coleta de fezes e visualização dos ovos de *F. hepatica* na técnica de sedimentação. Mediante o prognóstico reservado e declínio do animal foi realizada eutanásia. Na necropsia, o fígado estava amarelado com áreas friáveis, aumentado de volume nos lobos laterais direito, e os esquerdos diminuídos e firmes, dilatação e espessamento de ductos biliares, e ao corte intensa quantidade de parasitos compatíveis com *F. hepatica*. Na microscopia, observou-se ductos biliares distendidos com parede espessada e deposição de tecido conjuntivo fibroso intenso, degeneração de hepatócitos e bilestase multifocais intensos, hepatite eosinofílica e neutrofílica, discreta quantidade de cristais birrefringentes e amarelados intraductais. Portanto, baseados no histórico, região endêmica de fasciolose e manejo extensivo a base de *Brachiaria* spp., assim como nos achados clínico patológicos, conclui-se um quadro de fotossensibilização hepatógena por *Brachiaria* spp. associada e agravada por hepatite parasitária por *F. hepatica*.

**Palavras-chave:** Hepatopatia, *Fasciola hepatica*, emagrecimento progressivo, ruminantes, fotodermatite





## **HEPATOGENOUS PHOTSENSITIVITY BY *Brachiaria* spp. ASSOCIATED WITH FASCIOSIS IN NELORE COW: CASE REPORT**

Hepatogenous photosensitivity by *Brachiaria* spp. is a common intoxication in ruminants due to the deposition of steroidal saponins that leads to cholestasis and accumulation of the photodynamic pigment, phylloerythrin, in the peripheral circulation. Liver injury becomes more pronounced when associated with parasitism by *Fasciola hepatica*, with loss of functionality and canalicular obstructions. The objective is to report a case of hepatogenous photosensitivity by *Brachiaria* spp. in cow associated with fasciolosis, attended by the Veterinary Hospital of the Federal University of Espírito Santo. The six-year-old animal, Nelore, raised in an extensive regimen of *Brachiaria* spp., in Jerônimo Monteiro/ES, with a history of dermatitis without response to treatment. Clinically had multifocal dermatitis with intense necrosis and serosanguineous exudation, with dry and fibrous areas that were multifocal to coalescing, predominantly in the head, region: submandibular; semimembranosus and semitendinosus muscle; tarsal metatarsal and femorotibiopatellar joint; axillary and tail. The blood count revealed hypochromic normocytic anemia, hyperproteinemia, and neutrophilic leukocytosis. The biochemical profile showed increased values of total protein, globulins, aspartate aminotransferase, alkaline phosphatase, creatine kinase, gamma-glutamyl transferase, direct and indirect bilirubin and urea. A liver biopsy was performed showing intense multifocal degeneration of hepatocytes, mild multifocal ductal hyperplasia and moderate periportal fibrosis.



Fasciolosis was confirmed by collecting feces and visualizing *F. hepatica* eggs in the sedimentation technique. Due to the reserved prognosis and decline of the animal, euthanasia was performed. At necropsy, the liver was yellowish with friable areas, increased volume in the right lateral lobes, and the left ones were reduced and firm, dilatation and thickening of the bile ducts, and when cutting an intense number of parasites compatible with *F. hepatica*. Microscopy showed distended bile ducts with a thickened wall and deposition of intense fibrous connective tissue, degeneration of hepatocytes and intense multifocal bile stasis, eosinophilic and neutrophilic hepatitis, and a slight amount of intraductal birefringent and yellowish crystals. Therefore, based on the history, endemic region of fasciolosis and extensive management based on *Brachiaria* spp., as well as on the clinical and pathological findings, a picture of hepatogenous photosensitization by *Brachiaria* spp. associated and aggravated by parasitic hepatitis caused by *F. hepatica*.

**Keywords:** Liver disease, *Fasciola hepatica*, progressive weight loss, ruminants, photodermatitis



## **GRAU DE PERCEPÇÃO DE RISCO BIOLÓGICO PARA O VÍRUS DA DIARREIA VIRAL BOVINA (BVDV) E HERPESVÍRUS BOVINO TIPO 1 (BOHV-1), SEGUNDO O TAMANHO DO SISTEMA DE PRODUÇÃO LEITEIRO**

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Percepção de risco é a capacidade de identificar os riscos presentes em um ambiente, sendo diretamente influenciada pela cultura, experiência prévia, localização regional, grau de instrução, interferência da mídia e canais de comunicação. O objetivo principal deste estudo foi avaliar a percepção de risco biológico para o BVDV e BoHV-1 em rebanhos da região dos Campos Gerais do Paraná. Este estudo foi desenvolvido em parceria estabelecida pela Frísia Cooperativa Agroindustrial e USP, e os custos subsidiados pelo Programa Mais Leite Saudável (MAPA). Foram elaborados e aplicados formulários de análise de risco biológico contendo 10 questões, em 69 sistemas de produção de leite majoritariamente compostos por fêmeas Holandesas (91,30%; 63/69), com média de 183 vacas de lactação, produção individual de 32 litros/dia, CCS de 221.000 células/mL, e produção regional total de 460.745 de leite por dia. Os



dados captados foram distribuídos de acordo com os quartis do número de vacas em lactação, classificadas como pequenas, médias e grandes (abaixo de 60; 61-200; acima de 200 animais, respectivamente). Foram consideradas 8/10 questões de percepção de risco, o qual foi pontuada (0-10) e categorizada em níveis: alto ( $\geq 50$  pontos), médio (40 pontos) e baixo ( $\leq 30$  pontos). As bezerras e as vacas em lactação foram as categorias mais citadas (55,84 %; 43/77 / 22,07%; 17/77, respectivamente) quanto à predisposição às viroses. A percepção de proteção da fazenda aos agentes infecciosos do estudo estava associada diretamente ao grau de percepção de risco do produtor (P Valor  $< 0.0001$ ). A baixa percepção de risco estava associada ao tamanho da propriedade (P valor  $< 0.031$ ), e as propriedades grandes apresentavam menores chances de baixa percepção de risco (0,09; 0,011 - 0,915; 95% IC) em relação às propriedades pequenas (0,973; 0,301-3,144; 95% IC) Do ponto de vista sanitário esses resultados são úteis para direcionar futuras ações (treinamentos, dia de campo, planos de ação) nos sistemas produtivos que mais requerem atenção, bem como assistir às propriedades disseminando conhecimento sobre as enfermidades adotando de maneira assertiva medidas de biosseguridade para o controle dos vírus.

**Palavras-chaves:** risco biológico, sanidade; análise de risco



## **DEGREE OF PERCEPTION OF BIOLOGICAL RISK FOR BOVINE VIRAL DIARRHEA VIRUS (BVDV) AND BOVINE HERPESVIRUS TYPE 1 (BOHV-1), ACCORDING TO THE SIZE OF THE DAIRY PRODUCTION SYSTEM**

Risk perception is the ability to identify the risks present in an environment, and is directly influenced by culture, previous experience, regional location, level of education, media interference and communication channels. The main objective of this study was to evaluate the perception of biological risk for BVDV and BoHV-1 in herds in the Campos Gerais region of Paraná. This study was developed in partnership established by Frísia Cooperativa Agroindustrial and USP, and costs subsidized by the More Healthy Milk Program (MAPA). Biological risk analysis forms containing 10 questions were elaborated and applied in 69 milk production systems mostly composed of Holstein cows (91.30%; 63/69) an average of 183 lactating cows, individual production of 32 liters/day, SCC of 221.000 cells/mL, and total regional production of 460.745 liters of milk per day. Data captured were distributed according to quartiles of the number of lactating cows, classified as small, medium, and large (below 60; 61-200; above 200 animals, respectively). We scored eight out of ten risk perception questions that were scored (0-10) and categorized into levels: high ( $\geq 50$  points), medium (40 points), and low ( $\leq 30$  points). Calves and lactating cows were the most cited categories (55.84 %; 43/77 / 22.07%; 17/77, respectively) regarding predisposition to viruses. The perception of farm protection to infectious agents in the study was directly associated with the degree of producer risk perception (P value $<0.0001$ ). Low



risk perception was associated with farm size ( $P$  value  $< 0.031$ ), and large farms had lower chances of low risk perception (0.09; 0.011 - 0.915; 95% CI) compared to small farms (0.973; 0.301-3.144; 95% CI). From an animal health point of view, these results are useful to direct future actions (training, field days, action plans) in the productive systems that most require attention, as well as to assist farms by disseminating knowledge about diseases and assertively adopting biosecurity measures to control viruses.

**Keywords:** biological risk, animal health; risk analysis



## **IgG E ANTICORPOS ESPECÍFICOS CONTRA OS ENTEROPATÓGENOS NO COLOSTRO DE VACAS GIROLANDO**

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O cruzamento Holandês x Gir (Girolando) contribui com 80% da produção de leite no Brasil, devido ao seu perfil de resistência e adaptabilidade às condições climáticas dos países subtropicais e tropicais. Estudos específicos envolvendo a raça Girolando são escassos, e em nosso conhecimento não existem pesquisas sobre a qualidade imunológica do colostro bovino de novilhas e vacas Girolando. O objetivo principal desta pesquisa foi determinar a qualidade do colostro de vacas Girolanda com histórico de vacinação no pré-parto contra diarreia neonatal, por meio do colostrômetro, refratômetro brix, colostro *balls*, além da avaliação do teor de IgG total e anticorpos específicos contra os enteropatógenos, utilizando-se ELISA *sandwich* e kits comerciais de ELISA competição (Bio-X *Diagnostics* S.A.). Foram incluídas 18 fêmeas primíparas e 33 multíparas Girolando. O colostro foi obtido até 2 horas pós-parto. Foi registrado o volume de colostro (litros), e a massa de IgG calculada multiplicando-se volume x concentração determinada pelo padrão-ouro (ELISA *sandwich*). IgG total e específica contra os enteropatógenos foram mensurados no soro das vacas e no colostro centrifugado. O volume total de colostro



produzido pelos animais foi  $2,56 \pm 0,30$  Kg, a qualidade imunológica avaliada através do ELISA *sandwich* foi  $100,2 \pm 5,32$ , colostrômetro  $74,06 \pm 6,01$  mg/ml, colostro *balls*  $1.051 \pm 2,64$  e Brix  $25,08 \pm 0,77\%$ . O cálculo da massa de IgG foi  $207,16 \pm 23,14$ . Primíparas produziram  $2,75 \pm 0,76$  Kg de colostro, ELISA  $98,12 \pm 9,44$ , colostrômetro  $61,42 \pm 10,57$ , Brix  $23,29\% \pm 1,69$ , colostro *balls*  $1.045 \pm 4,62$  e o valor da massa de IgG  $207,16 \pm 17,55$ . Em múltíparas o ELISA foi  $101,46 \pm 6,51$ , colostrômetro  $79,96 \pm 7,18$  mg/mL, brix  $26,03\% \pm 0,77$ , colostro *balls*  $1.054 \pm 3,14$  e massa de IgG foi  $274,74 \pm 17,90$ . Os anticorpos específicos contra *Coronavírus*, *Rotavírus*, *Clostridium* (CP Alpha, Beta e épsilon) e *E. coli* apresentaram diferença ao longo do tempo ( $P \leq 0.0001$ ) apresentando variações entre os momentos estudados, alguns patógenos aumentaram ao longo do estudo, como por exemplo CP beta e épsilon. Os animais foram desafiados em algum momento no pós-parto apresentando queda na imunidade, ocasionando aumento dos anticorpos específicos. Mais estudos devem ser realizados comparando vacas mestiças com Holandesas.

**Palavras-chave:** Imunidade, Vacas mestiças, avaliação colostrálica, paridade, transferência de imunidade passiva





## **SPECIFIC IgG AND ANTIBODIES AGAINST THE HOLSTEIN X GIR CROSSBRED COLOSTRUM ENTEROPATHOGENS**

The Holstein x Gir crossbreed (Girolando) contributes with 80% of milk production in Brazil, due to its resistance profile and adaptation to the climatic conditions of subtropical and tropical countries. Studies specifically for the Girolando breed are scarce, and in our knowledge, there are none about immune quality of the colostrum of Girolando heifers and cows. The main objective of this research was to determine the quality of colostrum of Girolando cows with history of pre-calving vaccination against neonatal diarrhea, through colostrometer, Brix refractometer, colostrum balls, besides evaluation of total IgG level and specific antibodies against enteropathogens, using ELISA sandwich and commercial ELISA competition kits (Bio-X Diagnostics S.A.). 18 primiparous and 33 multiparous Girolando females were included. Colostrum was obtained up to 2 hours postpartum. The volume of colostrum (liters) and the IgG mass calculated by multiplication volume x concentration determined by gold-standard procedure (ELISA sandwich) were recorded. Total and specific IgG against enteropathogens were measured in cow serum and in centrifuged colostrum. The total volume of colostrum produced was  $2,56\pm 0,30$ kg, the immune quality evaluated through ELISA sandwich was  $100,2\pm 5,32$ , colostrometer  $74,06\pm 6,01$ mg/ml, colostrum balls  $1.051\pm 2,64$  and Brix  $25,08\pm 0,77\%$ . The IgG mass calculation was  $207.16\pm 23.14$ . Primiparous produced  $2.75\pm 0.76$  kg of colostrum, ELISA  $98.12\pm 9.44$ , colostrometer  $61.42\pm 10.57$ , Brix  $23.29\pm 1.69\%$ , colostrum balls  $1.045\pm 4,62$  and IgG mass value of  $207,16\pm 17,55$ . In multiparous,



ELISA was  $101,46 \pm 6,51$ , colostrometer  $79.96 \pm 7,18 \text{mg/mL}$ , Brix  $26,03 \pm 0.77\%$ , colostrum balls  $1.054 \pm 3.14$  and IgG mass was  $274.74 \pm 17.90$ . Specific antibodies against *Coronavirus*, *Rotavirus*, *Clostridium* (CP Alpha, Beta and Epsilon) and *E. coli* showed differences over time ( $P \leq 0.0001$ ) presenting variation between moments of studies, some pathogens increased over the study, such as CP beta and epsilon. The animals were challenged at some point in the postpartum period, decreasing immunity consequently increasing specific antibodies. More studies should be carried out comparing crossbred with Holstein cows.

**Keywords:** Immunity, crossbred cows, colostrum evaluation, number of births, passive immunity transfer



## **IMPACTO DA EXPRESSÃO DE PD-1 E CTLA-4 EM LINFÓCITOS NA SUSCEPTIBILIDADE E OCORRÊNCIA DE MASTITE DURANTE O PERÍODO PÓS-PARTO EM VACAS PRIMÍPARAS**

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O objetivo deste estudo foi avaliar a influência do PD-1 e CTLA-4 na susceptibilidade e ocorrência de mastite no pós-parto em novilhas. Foram examinadas trinta novilhas da raça Holandesa avaliadas em cinco momentos: quinze dias antes do parto (M1), no dia do parto M2, sete dias (M3), quinze (M4) e trinta dias após o parto (M5). No M1 apenas sangue foi coletado. A partir do M2, leite e sangue foram coletados para avaliação hematológica e avaliação da ocorrência de mastite. No M1, o sangue coletado foi destinado para separação das células mononucleares do sangue periférico (PBMC). Por meio deste, foi realizada a imunofenotipagem pela citometria de fluxo com anticorpos específicos. O leite foi avaliado pelos testes da caneca do fundo escuro,



*California Mastitis Test* (CMT), contagem de células somáticas microscópica diferencial (CCSMD) e MALDI-ToF MS. A análise estatística foi realizada utilizando o software SAS 9.4 (SAS *Institute*). Vários modelos logísticos multivariáveis foram usados para avaliar a relação entre diferentes populações de células e distribuição binária para a variável resultado. Foram isolados 25 patógenos em 64,37% dos quartos mamários avaliados. Em relação aos resultados imunológicos, foi possível observar influência da PD-1 e CTLA-4 na ocorrência de mastite. Por meio da análise de estimativa de probabilidade, foi possível observar que houve uma tendência na ocorrência de mastite ( $P = 0,07$ ) à medida que ocorreu um aumento de linfócitos PD-1. O mesmo foi observado quando houve uma tendência para aumento dos linfócitos CTLA-4 ( $P = 0,09$ ). Houve diferença significância na contagem total de PD-1 e CTLA-4 ( $P = 0,05$  e  $P = 0,04$ ), mostrando que o aumento de ambos afeta a ocorrência de mastite. A expressão de PD-1 e CTLA-4 nos LT influenciaram para maior ocorrência de mastite. O aumento de casos foi de 1,098 sob a influência do PD-1 e 1,067 sob a influência do CTLA-4. Com isso, observou-se que quanto maior a expressão de ambos no período que antecede o parto, maior foi a ocorrência de mastite subclínica no pós parto.

**Palavras-chaves:** Mastite. Bovinos. Citometria de fluxo. Imunofenotipagem



## **IMPACT OF PD-1 AND CTLA-4 EXPRESSION IN LYMPHOCYTES ON SUSCEPTIBILITY AND OCCURRENCE OF MASTITIS DURING THE POSTPARTUM PERIOD IN PRIMIPAROUS COWS**

The objective of this study was to evaluate the influence of PD-1 and CTLA-4 on susceptibility and occurrence of postpartum mastitis in heifers. Thirty Holstein heifers from two dairy farms were examined. The animals were evaluated in five different moments: fifteen days before parturition (M1), on the day of parturition (M2), seven days (M3), fifteen (M4) and thirty days after parturition (M5). At M1 there was immunological evaluation and only blood was collected. From M2, milk and blood were collected for hematological evaluation and evaluation of the occurrence of mastitis. At M1, the blood collected was destined for peripheral blood mononuclear cell (PBMC) separation. Through this, immunophenotyping was performed by flow cytometry with specific antibodies. Milk was evaluated by dark-bottom mug test, California Mastitis Test (CMT), differential microscopic somatic cell count (DMSC) and MALDI-ToF MS. Statistical analysis was performed using SAS 9.4 software (SAS Institute). Several multivariable logistic models were used to evaluate the relationship between different cell populations and binary distribution for the outcome variable. Twenty-five pathogens were isolated in 64.37% of the mammary gland evaluated. For the immunological results, it was possible to observe influence of PD-1 and CTLA-4 on the occurrence of mastitis. By analysis of probability estimation, it was possible to observe that there was a tendency in the occurrence of mastitis ( $P = 0.07$ ) as



there was an increase in PD-1 lymphocytes. The same was observed when there was a tendency for an increase in CTLA-4 lymphocytes ( $P = 0.09$ ). There was a significant difference in total PD-1 and CTLA-4 counts ( $P = 0.05$  and  $P = 0.04$ ), showing that an increase in both affects the occurrence of mastitis. The expression of PD-1 and CTLA-4 in the LT influenced for higher occurrence of mastitis. The increase of cases was 1.098 when influenced by PD-1 and 1.067 when influenced by CTLA-4. Thus, it was observed that the higher the expression of both in the period before calving, the higher was the occurrence of subclinical mastitis in the post calving period.

**Keywords:** Mastitis. Bovine. Flow cytometry. Immunophenotyping



## **INCIDÊNCIA DE ONFALOPATIAS EM BEZERROS DA RAÇA GIROLANDO CRIADOS NO TRÓPICO ÚMIDO AMAZÔNICO**

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As onfalopatias estão entre as infecções mais comuns que acometem bezerros neonatos, por isso a atenção deve ser redobrada na ingestão do colostro no tempo, quantidade e qualidade ideal, o que auxilia na prevenção de infecções, as quais o bezerro está suscetível. Destarte, objetivou-se com este trabalho avaliar incidência de onfalopatias em bezerros da raça girolando criados no trópico úmido amazônico. O estudo foi realizado em uma fazenda localizada no município de Paragominas, no período de dezembro de 2021 até julho de 2022, onde foram avaliados 80 bezerros (machos e fêmeas) da raça Girolando. Somente foram incluídos no estudo, bezerros nascidos de partos eutócicos monitorizados. Os bezerros foram separados de suas mães, tempestivamente, após o nascimento. Os animais eram acompanhados diariamente, desde o nascimento até os 60 dias de idade, e qualquer alteração no estado de saúde no



animal era identificada e o diagnóstico definitivo estabelecido. Os animais eram colostrados utilizando-se colostro descongelado em banho-maria, à temperatura de 35 a 40°C, grau Brix igual a 25%, ofertados em um volume de 10% do peso corpóreo nas primeiras duas horas pós-nascimento. Quando o colostro descongelado não atingia esse percentual, era enriquecido com colostro artificial em pó, consoante determinação do fabricante até atingir Brix igual a 25%. A prevenção de doenças do umbigo era feita com imersão do coto umbilical em solução de iodo 10%, diariamente até a mumificação e queda do cordão umbilical externo. No presente estudo foi observada incidência de 8% de animais acometidos com onfalopatias, o que está acima dos valores considerados padrão-ouro nacionais para a criação de bezerras em fase de aleitamento, em que se recomenda a taxa de morbidade aceitável para infecção umbilical menor que 5% na fase de aleitamento. Possivelmente, esses maiores valores estejam relacionados às condições de criação dos bezerros, em bezerreiros tropicais, de chão coberto por pedregulhos, o que não favorecia um manejo higiênico adequado das instalações, tampouco havia adoção de medidas de biossegurança no bezerreiro.

**Palavras-chave:** bovino, zebuínos, neonato, umbigo, doença





## **INCIDENCE OF OMPHALOPATHIES IN GIROLANDO CALVES BREEDED IN THE AMAZON MOIST TROPIC**

Omphalopathies are among the most common infections that affect newborn calves, so attention should be redoubled in colostrum intake at the ideal time, quantity and quality, which helps in the prevention of infections, to which the calf is susceptible. Thus, the objective of this work was to evaluate the incidence of omphalopathies in girolando calves raised in the humid tropics of the Amazon. The study was carried out on a farm located in the municipality of Paragominas, from December 2021 to July 2022, where 80 Girolando calves (males and females) were evaluated. Only calves born from monitored eutocic deliveries were included in the study. The calves were separated from their mothers, timely, after birth. The animals were monitored daily, from birth to 60 days of age, and any change in the animal's health status was identified and the definitive diagnosis established. The animals were colostrated using thawed colostrum in a water bath, at a temperature of 35 to 40°C, Brix degree equal to 25%, offered in a volume of 10% of body weight in the first two hours after birth. When the thawed colostrum did not reach this percentage, it was enriched with artificial colostrum powder, as determined by the manufacturer, until it reached a Brix equal to 25%. The prevention of navel diseases was performed by immersion of the umbilical stump in a 10% iodine solution, daily until mummification and the external umbilical cord fell off. In the present study, an incidence of 8% of animals affected with omphalopathies was observed, which is above the values



considered national gold standard for the rearing of suckling calves, in which an acceptable morbidity rate for umbilical infection lower than 5% in the lactation phase. Possibly, these higher values are related to the conditions of rearing calves, in tropical calves, with a floor covered by boulders, which did not favor an adequate hygienic management of the facilities, nor was there the adoption of biosecurity measures in the calf.

**Keywords:** bovine, zebu, neonate, navel, disease



## **ÍNDICE DE TÔNUS VASOVAGAL E FREQUÊNCIA CARDÍACA EM NOVILHAS SUBMETIDAS A SEDAÇÃO COM XILAZINA**

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Uma das propriedades do coração é o automatismo, gerando os próprios potenciais de ação, culminando na contração miocárdica. O Índice do Tônus Vasovagal (iTVV) obtido a partir de traçados eletrocardiográficos em curtos períodos representa a variabilidade da frequência cardíaca (VFC) no domínio do tempo, considerando as variações da frequência cardíaca. Dessa forma, o objetivo do trabalho foi avaliar o Índice do Tônus Vasovagal em novilhas submetidas a procedimentos anestésicos. Foram avaliados traçados eletrocardiográficos de nove novilhas da raça nelore, que passaram por um procedimento de biópsia de glândula mamária, na Superintendência Unidade Hospitalar Veterinária Universitária, em três momentos, basal, trans-cirúrgico e pós-operatório imediato. Os animais foram sedados com Xilazina 0,2 mg/kg e controle da dor com Meloxicam 0,22 mg/kg. Para a realização do iTVV utilizou-se o logaritmo neperiano da variância dos valores de vinte intervalos R-R consecutivos, na derivação DII do traçado. Para a obtenção da frequência



cardíaca foi considerado o intervalo entre dois R-R adjacentes. Os dados foram submetidos ao teste de normalidade Kolmogorov-Smirnov e as variáveis em paramétricas, descritas em média e desvio padrão, foram submetidas ao teste ANOVA e ao teste de Tukey. Os valores médios de iTVV e frequência cardíaca foram, respectivamente, no momento basal  $6,094 \pm 0,706$  e  $67,56 \pm 5,31$ , trans-cirúrgico  $7,338 \pm 1,022$  e  $55,78 \pm 5,02$ , e pós-operatório  $6,142 \pm 1,169$  e  $58,17 \pm 8,26$ . Ao comparar os momentos, constatou-se diferenças dos valores médios obtidos de iTVV entre os momentos basal e pós-operatório em relação ao trans-cirúrgico ( $p=0,0202$ ). Já, ao comparar os valores médios da frequência cardíaca constatou-se diferenças entre os momentos trans-cirúrgico e pós-operatório em relação ao basal ( $p=0,0001$ ). Sedativos da classe agonistas alfa-2, como a xilazina, têm efeito importante em receptores pré-sinápticos, causando uma redução da liberação de noradrenalina e redução da atividade simpática. Dessa forma, o sedativo, causa um desbalanceamento do sistema autonômico alterando a frequência cardíaca durante o período de ação e alterando a VFC durante os procedimentos. Dessa forma evidenciando os efeitos sistêmicos e cardíacos causados pela xilazina, com o aumento do iTVV e redução da frequência cardíaca.

**Palavras-chave:** eletrocardiograma, frequência cardíaca, anestesia.



## **VASOVAGAL TONUS INDEX AND HEART RATE IN HEIFERS SUBMITTED TO SEDATION WITH XYLAZINE**

One of the properties of the heart is automatism, generating its own action potentials, culminating in myocardial contraction. The Vasovagal Tonus Index (iTVV) obtained from electrocardiographic tracings in short periods represents the heart rate variability (HRV) in the time domain, considering heart rate variations. Thus, the objective of this study was to evaluate the Vasovagal Tonus Index in heifers submitted to anesthetic procedures. Electrocardiographic tracings of nine Nelore heifers were evaluated, which underwent a mammary gland biopsy procedure, at the Superintendence University Veterinary Hospital Unit, in three moments, baseline, trans-surgical and immediate postoperative. The animals were sedated with Xylazine 0.2 mg/kg and pain control with Meloxicam 0.22 mg/kg. To perform the iTVV, the Neperian logarithm of the variance of the values of twenty consecutive R-R intervals was used, in the DII derivation of the tracing. To obtain the heart rate, the interval between two adjacent R-Rs was considered. The data were submitted to the Kolmogorov-Smirnov normality test and the parametric variables, described as mean and standard deviation, were submitted to the ANOVA test and the Tukey test. The mean values of iTVV and heart rate were, respectively, at baseline  $6.094 \pm 0.706$  and  $67.56 \pm 5.31$ , trans-surgical  $7.338 \pm 1.022$  and  $55.78 \pm 5.02$ , and postoperatively  $6.142 \pm 1.169$  and  $58.17 \pm 8.26$ . When comparing the moments, differences in the



mean values obtained for iTVV were observed between the baseline and postoperative moments in relation to the trans-surgical period ( $p=0.0202$ ). On the other hand, when comparing the mean values of heart rate, differences were found between the trans-surgical and postoperative moments in relation to the baseline ( $p=0.0001$ ). Alpha-2 agonist class sedatives, such as xylazine, have an important effect on presynaptic receptors, causing a reduction in noradrenaline release and a reduction in sympathetic activity. In this way, the sedative causes an imbalance of the autonomic system, altering the heart rate during the period of action and altering the HRV during the procedures. Thus, evidencing the systemic and cardiac effects caused by xylazine, with an increase in iTVV and a reduction in heart rate.

**Keywords:** Electrocardiogram, heart rate, anesthesia



## **INFECÇÃO PELO VÍRUS DA DIARREIA VIRAL BOVINA (BVDV) EM REBANHOS DA REGIÃO DOS CAMPOS GERAIS PARANAENSE**

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O BVDV é um vírus produtivo e reprodutivo, que causa grande impacto econômico nos sistemas de produção leiteiros. Este estudo foi desenvolvido em parceria USP e Frísia Cooperativa Agroindustrial, sendo os custos subsidiados pelo Programa Mais Leite Saudável (MAPA). O objetivo deste resumo é apresentar os dados epidemiológicos da infecção pelo BVDV na região dos Campos Gerais no Paraná. Amostras do tanque de leite foram coletadas de 289 fazendas cooperadas, entre agosto de 2020 a janeiro de 2022, para a investigação do vírus à nível rebanho pelo qPCR. Destas, 68 foram selecionados por conveniência para pesquisa de fêmeas persistentemente infectadas (PI), através do teste ELISA antígeno a partir de tecido auricular. A população-alvo foi composta pela última geração do rebanho, fêmeas que pariram machos/natimortos. Recomendou-se repetir os exames nos positivos, com intervalo mínimo de 21 dias, além da testagem das mães e avós. Conforme resultados



dos testes, os rebanhos foram divididos em quatro categorias: 1 – Negativo para ambos testes; 2-qPCR positivo e ELISA Ag negativo; 3-qPCR negativo e ELISA Ag positivo e 4-Positivo para ambos. Ao todo foram realizados 2.902 qPCR e 23.466 testes de ELISA antígeno. Assim, 76,12% (220/289) das propriedades foram consideradas negativas, 23,87% (69/289) positivas em pelo menos um teste qPCR. 41,17% (28/68) das fazendas, tiveram pelo menos um resultado positivo para ELISA Ag, enquanto 58,83% foram negativas. Quanto às categorias, 41,17% (28/68) propriedades foram consideradas livres do BVDV (categoria 1); 17,64% (12/68) incluídas na categoria 2; 13,23% (9/68) na categoria 3 e 27,94% (19/68) classificados verdadeiramente como positivos - categoria 4. Quanto às práticas de vacinação em 62 propriedades: 72,58% (45/62) dos produtores vacinavam contra os vírus reprodutivos e 27,41% (17/62) não vacinavam. Cinco produtores (8,06%; 5/62) reportaram o uso de até duas marcas comerciais diferentes para vacinação de doenças reprodutivas, a depender da disponibilidade do mercado. Apenas 4 (4/62, 6,45%) utilizavam vacinas vivas modificadas. A frequência do manejo vacinal variou de 1 até 4 vezes ao ano. Este levantamento epidemiológico demonstra a importância da implementação de programas de controle contra o BVDV, o que inclui práticas de biossegurança e revisão dos protocolos de vacinação.

**Palavras-chaves:** vírus reprodutivo, vacinação, ELISA antígenos





## **BOVINE VIRAL DIARRHEA VIRUS (BVDV) INFECTION IN HERDS IN THE CAMPOS GERAIS REGION OF PARANAENSE**

BVDV is a productive and reproductive virus that causes great economic impact in dairy production systems. This study occurred in partnership with USP and Frisia Agroindustrial Cooperative, with costs subsidized by the More Healthy Milk Program (MAPA). The aim of this abstract is to present the epidemiological data of BVDV infection in the Campos Gerais region of Paraná. Milk tank samples were collected from 289 cooperative farms from August 2020 to January 2022, to investigate BVDV infection at herd level through qPCR. Out of these farms, 68 were selected by convenience to search for persistently infected females (PI) through antigen ELISA test from ear tissue. The target population was composed of the herd's last generation, females that gave birth to males and stillbirths. It was recommended to repeat the tests on positive animals, with a minimum interval of 21 days, in addition to testing the dams and granddams. According to the results of tests, the herds were divided into four categories: 1 - Negative for both tests; 2-qPCR positive and ELISA Ag negative; 3-qPCR negative and ELISA Ag positive and 4-Positive for both. In all, 2,902 qPCR and 23,466 antigen ELISA tests were performed. Thus, 76.12% (220/289) of the properties were considered negative and 23.87% (69/289) positive in at least one qPCR test. To PI survey, 41.17% (28/68) of the farms had at least one positive result for ELISA Ag, while 58.83% were negative. 41.17% (28/68) properties were considered free of BVDV (category 1); 17.64% (12/68) in category 2; 13.23% (9/68) in category 3 and 27.94% (19/68) were truly classified positive - category 4. Regarding vaccination



practices, 62 of the 68 properties: 72.58% (45/62) of the producers vaccinated against reproductive viruses and 27.41% (17/62) did not vaccinate. Five producers (8.06%; 5/62) reported the use up to two different brands for vaccination of reproductive diseases, depending on market availability. Only 4 (4/62, 6.45%) used modified live vaccines. The frequency of vaccine management ranged from 1 to 4 times a year. This epidemiological survey demonstrates the importance of implementing control programs against BVDV, which includes biosecurity practices and review of vaccination protocols.

**Keywords:** reproductive virus, vaccination, antigen ELISA



## **INFECÇÃO PELO VÍRUS DA LEUCEMIA BOVINA E INFLUÊNCIA DA CARGA PROVIRAL NO PERFIL HEMATOLÓGICO, BIOQUÍMICO E INFLAMATÓRIO DE NOVILHAS DURANTE O PERÍODO DE TRANSIÇÃO**

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O objetivo da pesquisa foi avaliar o impacto do Vírus da Leucemia Bovina (*Bovine Leukemia Virus - BLV*) sobre o perfil hematológico, bioquímico, inflamatório e metabolismo energético em novilhas leiteiras naturalmente infectadas no período de transição. O rebanho foi triado para determinação da prevalência de anticorpos específicos anti-BLV por ELISA. Após a triagem inicial e confirmação pela técnica de reação da polimerase em cadeia (PCR), 24 novilhas prenhas das raças Holstein e Jersey foram distribuídas em 2 grupos experimentais, composto por novilhas positivas BLV +, (n=12); e negativas BLV -, (n=12). Amostras de sangue foram colhidas semanalmente (-3, -2, -1, parto, +1, +2 e +3 semanas) e avaliadas para determinação de perfil hematológico, bioquímico, hepático (AST, GGT) inflamatório (Fibrinogênio, HP) e energético (BHB, triglicerídeos e colesterol). Foi realizada a pesquisa de DNA proviral por



avaliação da reação da polimerase em cadeia (ddrPCR) nas semanas -3, parto e +3. Os animais foram monitorados diariamente por sistema automático de avaliação de ruminação. Foram levantados registros relativos à saúde, produção e qualidade do leite. Os animais foram ainda distribuídos em grupos com alta e baixa carga proviral, para avaliação dos mesmos parâmetros previamente descritos. O método de regressão logística foi usado para avaliação de inflamação, cetose e lesão hepática. A prevalência geral para anticorpos anti-BLV foi 57.25% (351/613), sendo em novilhas prenhes 38.7% (76/124). A infecção por BLV alterou o perfil hematológico das novilhas para CHCM ( $P=0.026$ ), bioquímico com a elevação nas concentrações da enzima AST ( $P=0.023$ ), além de alterar o perfil cinético dos triglicerídeos entre os grupos ( $P=0.023$ ). A avaliação do perfil inflamatório revelou influência da infecção por BLV no fibrinogênio ( $P=0.043$ ), e aumento no risco de elevação da HP (OR= 4.959). As contagens leucocitárias de animais com alta carga viral foram maiores no grupo com alta carga viral ( $P=0.018$ ), especialmente por linfocitose ( $P=0.036$ ). Conclui-se que a infecção pelo BLV pode alterar o perfil hematológico, bioquímico e o metabolismo energético de novilhas no período de transição; além de aumentar as chances de inflamação. A carga viral pode impactar nas contagens leucocitárias, especialmente por predisposição à linfocitose.

**Palavras-chave:** Leucose, Carga proviral, Inflamação, Leucocitose, Linfocitose



## **BOVINE LEUKEMIA VIRUS INFECTION AND PROVIRAL LOAD INFLUENCES ON HEMATOLOGICAL, BIOCHEMICAL AND INFLAMMATORY PROFILE OF HEIFERS DURING THE TRANSITION PERIOD**

The aim of the study was to evaluate the impact of Bovine Leukemia Virus (BLV) on the hematological, biochemical, inflammatory and energy metabolism profile of naturally infected dairy heifers in the transition period. The herd was initially screened to determine the prevalence of specific anti-BLV antibodies by the ELISA technique. After initial serological screening and confirmation by polymerase chain reaction (PCR) 24 pregnant Holstein and Jersey heifers were distributed in 2 experimental groups, composed of positive BLV + heifers, (n=12); and BLV group – negative heifers (n=12). Blood samples were collected weekly at periods -3, -2, -1, parturition, +1, +2 and +3 weeks and evaluated for hematological, hepatic biochemical (AST, GGT), inflammatory (Fibrinogen, HP) and energy (BHB, triglycerides and cholesterol). Proviral DNA screening was performed by digital evaluation of the polymerase chain reaction (ddrPCR). The animals were monitored daily through an automatic rumination assessment system. Records concerning health, production and milk quality of heifers were collected. The animals were also divided into groups with high and low proviral load, for evaluation of the same parameters previously described. For statistical analysis, the data were linearized and evaluated by the mixed linear model (Mixed model). The logistic regression method was used to assess inflammation, ketosis and liver damage. General herd prevalence for anti-BLV antibodies was 57.25% (351/613), with 38.7% (76/124) in pregnant heifers. BLV infection altered



the hematological profile of heifers for CHCM ( $P= 0.026$ ), biochemical, with the increase of AST enzyme concentrations ( $P= 0.023$ ), in addition to altering the kinetic profile of triglycerides between groups ( $P= 0.023$ ). The evaluation of the inflammatory profile revealed an influence of BLV infection on fibrinogen ( $P= 0.043$ ), and a significant increase in the risk of elevation of HP ( $OR= 4.959$ ). WBC counts from animals with high viral load were significantly higher in the high viral load group ( $P=0.018$ ), especially by lymphocytosis ( $P=0.036$ ). It is concluded that BLV infection can alter the hematological and biochemical profile and energy metabolism of heifers in the transition period; in addition to increasing the chances of inflammation. Viral load can impact WBC counts, especially by predisposing lymphocytosis.

**Keywords:** Leukosis, Proviral load, Inflammation, Leukocytosis, Lymphocytosis



## INFECÇÃO PELO VÍRUS DA LEUCOSE ENZOÓTICA BOVINA NA REGIÃO DE MONTES CLAROS, MG: RELATO DE CASO

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A leucose enzoótica bovina (LEB) é uma doença infectocontagiosa, imunossupressora e tem como agente etiológico um retrovírus pertencente ao gênero *Deltaretrovírus*, chamado de *Bovine Leukemia Virus* (BLV), que afeta primariamente o sistema linfóide, apresenta evolução crônica, com capacidade de ocasionar, após longos períodos, uma proliferação de linfócitos infectados evoluindo à formação de linfossarcomas, causando prejuízos econômicos consideráveis. Objetivou-se relatar os sinais clínicos e exames complementares em um caso de LEB, na região de Montes Claros, em Minas Gerais. Trata-se de uma vaca multípara da raça holandesa, com aproximadamente 5 anos. A queixa principal baseia-se na hiporexia, isolamento do animal, permanência em decúbito durante maior parte do dia, bem como, redução na produção de leite. Segundo o proprietário houve um surto anterior de pneumonia no qual dois animais vieram a óbito. No contexto do exame clínico, o animal apresentava apatia, mucosas hipocoradas, desidratação 5-8%, temperatura 42°C, edema periorcular, tremores musculares, secreção mucopurulenta nas narinas e taquipneia. Detectou-se, à palpação retal, uma estrutura firme, não-delimitável,



localizada no assoalho pélvico. Nos exames complementares, a AST, ALT, GGT, proteínas totais e frações, creatinina e cálcio total encontravam-se dentro dos valores de referência estabelecidos para espécie, bem como, o eritrograma não demonstrou alterações e nem detectou-se, morfologicamente, hemoparasitas ao exame direto em esfregaço sanguíneo. Em contrapartida, no leucograma, havia leucocitose, linfocitose e neutrofilia acentuadas (leucócitos totais 103.330/ $\mu\text{L}$  (4.000-12.000/ $\mu\text{L}$ ), linfócitos 85.739/ $\mu\text{L}$  (2.500-7.500/ $\mu\text{L}$ ) e neutrófilos 16.280/ $\mu\text{L}$  (600-4.000/ $\mu\text{L}$ ). O fósforo e a ureia encontraram-se acima dos valores de referência. Solicitou-se um teste ELISA para detecção de anticorpos contra o vírus da leucose bovina sendo o resultado positivo. Embora tenha sido instituída terapia de suporte e administração de antibiótico, anti-inflamatório e complexo vitamínico, o animal em questão veio a óbito. O exame físico, associado ao leucograma sugestivo e a sorologia permitiram o diagnóstico da LEB, provavelmente associado a doenças oportunistas como a pneumonia. As medidas profiláticas para diminuir os riscos de transmissão da doença no rebanho foram devidamente instituídas.

**Palavras-Chave:** Doença infectocontagiosa, leucocitose, linfossarcoma, linfocitose





## BOVINE ENZOOTIC LEUKOSIS VIRUS INFECTION IN THE REGION OF MONTES CLAROS, MG: CASE REPORT

Enzootic bovine leukosis (EBL) is an infectious, immunosuppressive disease whose etiologic agent is a retrovirus belonging to the *Deltaretrovirus genus*, called *Bovine Leukemia Virus* (BLV), which primarily affects the lymphoid system, presents a chronic evolution, with the ability to cause, after long periods, a proliferation of infected lymphocytes evolving to the formation of lymphosarcomas, causing considerable economic losses. The objective was to report the clinical signs and complementary exams in a case of LEB, in the region of Montes Claros, in Minas Gerais. This is a multiparous Holstein cow, approximately 5 years old. The main complaint is based on hyporexia, isolation of the animal, remaining in recumbency for most of the day, as well as reduced milk production. According to the owner, there was a previous outbreak of pneumonia in which two animals died. In the context of the clinical examination, the animal presented apathy, pale mucous membranes, dehydration 5-8%, temperature 42°C, periorbital edema, muscle tremors, mucopurulent secretion in the nostrils and tachypnea. On rectal palpation, a firm, non-delimitable structure located on the pelvic floor was detected. In the complementary exams, AST, ALT, GGT, total proteins and fractions, creatinine and total calcium were within the reference values established for the species, as well as the erythrogram showed no alterations and morphologically, hemoparasites were not detected. direct blood smear examination. On the other hand, the leukogram showed marked leukocytosis, lymphocytosis and neutrophilia (total leukocytes 103,330/ $\mu$ L



(4,000-12,000/ $\mu$ L), lymphocytes 85,739/ $\mu$ L (2,500-7,500/ $\mu$ L) and neutrophils 16,280/ $\mu$ L (600-4,000/ $\mu$ L). Phosphorus and urea were above the reference values. An ELISA test was requested to detect antibodies against the bovine leukosis virus and the result was positive. Although supportive therapy and antibiotic, anti-inflammatory and vitamin complex administration were instituted, the animal in question died. The physical examination, associated with the suggestive leukogram and serology allowed the diagnosis of LEB, probably associated with opportunistic diseases such as pneumonia. Prophylactic measures to reduce the risk of disease transmission in the herd were duly instituted.

**Key words:** Infectious disease, leukocytosis, lymphosarcoma, lymphocytosis



## INFECÇÕES SIMULTÂNEAS POR *HISTOPHILUS SOMNI* E GAMAHERPESVÍRUS OVINO 2 EM BOVINOS DO SUL DO BRASIL

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*Histophilus somni* (HS) é uma bactéria gram-negativa que produz várias síndromes de doenças (manifestações respiratórias, reprodutivas, cardíacas e sistêmicas) que são coletivamente referidas como o complexo da doença de HS (CDHS). Gamaherpessvírus ovino 2 (OvGHV2) é membro do gênero Macavírus, faz parte do grupo dos vírus da febre catarral maligna (VFCM) e produz a febre catarral maligna (FCM) em vários hospedeiros mamíferos. Este relato apresenta os achados observados em dois surtos de CDHS em bovinos do sul do Brasil concomitantemente infectados por OvGHV2. O primeiro surto afetou 16,1% (5/31) de bovinos, Angus mestiços, com manifestações clínicas de salivação, incoordenação motora, decúbito lateral e morte súbita. Durante o segundo surto 1,6% (5/300) bovinos, nelore, morreram após apresentaram ataxia, prostração e decúbito lateral. Uma vaca de cada fazenda (animais A e B, respectivamente) foi



recebida para investigações patológicas e moleculares para determinar a possível causa da morte. Os principais achados patológicos observados na vaca A foram hemorragias pulmonares, miocárdicas, encefálicas com vasculite e pneumonia intersticial linfoplasmocítica com lesões vasculares proliferativas (LVP). Os principais achados patológicos observados em animal B foram broncopneumonia purulenta, miocardite hemorrágica e encefalite com vasculite multifocal e pneumonia intersticial linfoplasmocítica com LVP. PCR amplificou o DNA de *H. somni* de fragmentos do pulmão, traqueia e fígado da vaca A, e do miocárdio e cérebro da vaca B. Além disso, a imuno-histoquímica identificou antígenos intralésionais de um VFCM nos pulmões de ambas das vacas com pneumonia intersticial, enquanto a PCR confirmou que o MCFV amplificado era OvGHV2. Entretanto, *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma bovis*, vírus da diarreia viral bovina, vírus respiratório sincicial bovino, alfa herpesvírus bovino 1 e vírus da parainfluenza bovina 3 não foram identificados nos tecidos analisados por testes moleculares. Esses achados demonstram que ambos os animais de duas fazendas distintas foram infectados concomitantemente por *H. somni* e OvGHV2, sugerindo que esses organismos estavam associados ao desenvolvimento das manifestações clínicas observadas. Adicionalmente, a pneumonia intersticial foi associada ao OvGHV2, sugerindo que este patógeno deve ser considerado como um possível indutor de doença respiratória em bovinos, uma vez que outras causas comuns de pneumonia intersticial bovina não foram identificadas.

**Palavras-chave:** infecções concomitantes; febre catarral maligna; doença respiratória.



## **SIMULTANEOUS INFECTIONS BY *HISTOPHILUS SOMNI* AND OVINE AMMAHERPESVIRUS 2 IN CATTLE FROM SOUTHERN BRAZIL**

*Histophilus somni* (HS) is a gram-negative bacterium that produces several disease syndromes (respiratory, reproductive, cardiac, and systemic manifestations) that are collectively referred to as the HS disease complex (HSDC). Ovine gammaherpesvirus-2 (OvGHV2) is member of the Macavirus genus, forms part of the malignant catarrhal fever virus (MCFV) group and produces malignant catarrhal fever (MCF) in several mammalian hosts. This report presents the findings observed in two outbreaks of HSDS in cattle from Southern Brazil that were concomitantly infected by OvGHV2. The first outbreak affected 16.1% (5/31) of Mixed-breed Angus, cattle with clinical manifestations of salivation, uncoordinated gait, lateral decumbency, and sudden death. During the second outbreak 1.6% (5/300) of Nellore cattle died after presenting ataxia, prostration, lateral recumbency. One cow from each farm (animals A and B, respectively) was received for pathological and molecular investigations to determine the possible cause of death. The principal pathological findings observed in cow A were pulmonary, myocardial, encephalitic hemorrhages with vasculitis, and lymphoplasmacytic interstitial pneumonia with proliferating vascular lesions (PVL). The main pathological findings observed in cow B were purulent bronchopneumonia, hemorrhagic myocarditis and encephalitis with multifocal vasculitis as well as lymphoplasmacytic interstitial pneumonia with PVL. PCR assays amplified *H. somni* DNA from fragments of the lung, trachea,



and liver of cow A, and from the myocardium and brain of cow B. Additionally, immunohistochemistry identified intralesional antigens of a MCFV from the lungs of both cows with interstitial pneumonia, while PCR confirmed that the MVFC amplified was OvGHV2. However, *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma bovis*, bovine viral diarrhoea virus, bovine respiratory syncytial virus, bovine alphaherpesvirus 1, and bovine parainfluenza virus 3 were not identified in any of the tissues analyzed by molecular testing. These findings demonstrate that both animals from two distinct farms were concomitantly infected by *H. somni* and OvGHV2, suggesting that these organisms were associated with the development of the clinical manifestations observed. Furthermore, the interstitial pneumonia observed in both cows were associated with OvGHV2, suggesting that this pathogen should be considered as a possible inductor of respiratory disease in cattle.

**Keywords:** concomitant infections; malignant catarrhal fever; respiratory disease



## **INFLUÊNCIA DA DOENÇA RESPIRATÓRIA BOVINA E DIARREIA NA FASE DE ALEITAMENTO SOBRE O DESENVOLVIMENTO DA FUTURA VACA: LEVANTAMENTO DE DADOS**

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A ocorrência de diarreia e doença respiratória bovina (DRB) em bezerras resulta em impactos econômicos, reprodutivos e produtivos. O objetivo deste trabalho foi analisar o impacto da diarreia e DRB no desenvolvimento das bezerras e produção leiteira futura. O trabalho utilizou dados retrospectivos de fazenda leiteira localizada em Carambeí-PR. Todos os dados foram coletados de fêmeas bovinas da raça Holandesa, nascidas entre novembro de 2017 a novembro de 2018, sendo que estes dados estavam no software de gerenciamento (Dairy Plan C21 - GEA®). Para avaliação, as bezerras foram separadas em quatro grupos, sendo grupo Diarreia - bezerras com presença apenas de diarreia; grupo DRB – bezerras com presença apenas de DRB; grupo Diarreia + DRB – bezerras com presença de diarreia e DRB; grupo Saudáveis – bezerras sem diarreia ou DRB. No período do estudo nasceram 454 bezerras e o índice de mortalidade no aleitamento foi 9,47%. O grupo Diarreia apresentou maior taxa de morbidade ( $p < 0,0001$ ). O GMD mostrou diferenças entre o grupo Diarreia + DRB e o grupo



Saudáveis ( $p=0,001$ ). Animais do grupo DRB tiveram 2,35 mais chances de apresentar GMD abaixo de 1Kg ( $p<0,001$ ). Ademais, observou-se que bezerras com GMD acima de 1 kg tiveram 2,32 mais chances (IC 95% 1,39-3,88) de serem inseminadas com menos de um ano de idade ( $p=0,001$ ), quando comparados aos animais que apresentaram ganho médio diário abaixo de 1 kg. Maior frequência de animais do grupo Saudáveis apresentou produção de leite acima de 14.000 Kg na primeira lactação ( $p<0,0001$ ). O grupo Saudáveis apresentou maior produção de leite na primeira lactação (16.409,96 Kg) em relação ao grupo Diarreia (11.859,75 Kg) e grupo DRB (11.263,07 Kg) ( $p=0,007$ ). Com o trabalho concluímos que as doenças de bezerras no período de aleitamento têm impacto negativo no GMD e produção futura de leite das vacas em lactação.

**Palavras-chave:** desempenho; doenças; ganho de peso; impactos econômicos; produção de leite





## **THE INFLUENCE OF BOVINE RESPIRATORY DISEASE AND DIARRHEA IN THE LACTATION PHASE ON THE DEVELOPMENT OF THE FUTURE COW: DATA SURVEY**

The occurrence of diarrhea and bovine respiratory disease (RBD) in calves results in economic, reproductive and productive impacts. The objective of this work was to analyze the impact of diarrhea and DRB on calf development and future milk production. The work used retrospective data from a dairy farm located in Carambeí-PR. All data were collected from Holstein cows, born between November 2017 and November 2018, and these data were in the management software (Dairy Plan C21 - GEA®). For evaluation, the calves were separated into four groups, namely: Diarrhea Group - calves with only diarrhea; DRB group – calves with only DRB presence; Diarrhea + DRB Group – calves with diarrhea and DRB; Healthy group – calves without diarrhea or DRB. During the study period, 454 heifers were born and the lactation mortality rate was 9.47%. The Diarrhea group had the highest morbidity rate ( $p < 0.0001$ ). The ADG showed differences between the Diarrhea + DRB group and the Healthy group ( $p = 0.001$ ). The animals in the DRB group were 2.35 times more likely to have GDM below 1Kg ( $p < 0.001$ ). Furthermore, it was observed that calves with ADG above 1 kg were 2.32 times more likely (95% CI 1.39-3.88) to be inseminated at less than one year of age ( $p = 0.001$ ), when compared to animals that showed average daily gain below 1kg. Higher frequency of animals in the Healthy group presented milk production above 14,000 kg in the first lactation ( $p < 0.0001$ ). The Healthy group had higher milk production in the first lactation (16,409.96 kg) compared to the



Diarrhea group (11,859.75 kg) and the DRB group (11,263.07 kg) ( $p=0.007$ ). With the work we conclude that diseases of calves during the lactation period have a negative impact on ADG and on the future milk production of lactating cows.

**Keywords:** performance; disease; weight gain; economic impacts; milk production



## **INFLUÊNCIA DAS FASES DE LACTAÇÃO E DA MASTITE SUBCLÍNICA NAS CONCENTRAÇÕES DE PROTEÍNAS TOTAIS E ALBUMINA E ATIVIDADE DE GGT NO SORO LÁCTEO DE VACAS LEITEIRAS**

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O objetivo do estudo foi determinar as alterações causadas pela mastite subclínica na composição do soro lácteo, levando-se em consideração as variações destes componentes decorrente das alterações fisiológicas que ocorrem durante a lactação. Foram coletadas e avaliadas amostras de leite de quartos mamários sadios (CMT e microbiologia negativos) de vacas no 1º (G1, n=70), 2º (G2, n=37) e 3º (G3, n=147) trimestre de lactação, e de quartos mamários com mastite subclínica (CMT e microbiologia positivos) de vacas no 1º (G4, n=28), 2º (G5, n=21) e 3º (G6, n=113) trimestre de lactação. Foram analisados, no soro lácteo, as concentrações de proteínas totais (PT) e albumina, além da atividade de gama glutamil transferase (GGT), mediante leitura em espectrofotômetro semi-automático, no Laboratório de Análises Clínicas do HV da UEM (Campus de Umuarama). Os parâmetros foram submetidos à ANOVA e ao teste de Tukey ( $p < 0,05$ ). Foi possível determinar a média $\pm$ DP das variáveis



analisadas: 1) PT (g/dL) - G1:  $1,20 \pm 0,20$ ; G2:  $1,30 \pm 0,27$ ; G3:  $1,50 \pm 0,30$ ; G4:  $1,60 \pm 0,86$ ; G5:  $1,60 \pm 0,58$ ; G6:  $1,70 \pm 0,62$ ; 2) Albumina (g/dL) - G1:  $0,07 \pm 0,03$ ; G2:  $0,09 \pm 0,03$ ; G3:  $0,10 \pm 0,08$ ; G4:  $0,13 \pm 0,11$ ; G5:  $0,14 \pm 0,07$ ; G6:  $0,17 \pm 0,22$ ; 3) GGT (U/L) - G1:  $2518 \pm 991$ ; G2:  $2895 \pm 1725$ ; G3:  $3151 \pm 1297$ ; G4:  $3436 \pm 2925$ ; G5:  $3337 \pm 1563$ ; G6:  $3810 \pm 1095$ . Observou-se influência significativa das fases de lactação, com maiores concentrações de PT e albumina no G2 em relação ao G1 e no G3 em relação ao G1 e G2. Também observou-se maiores atividades, no soro lácteo, de GGT no G3 em relação ao G1. Verificou-se influência significativa da mastite subclínica, com maiores concentrações de PT e albumina no G4, G5 e G6 em relação ao G1, G2 e G3, respectivamente. Além disso, verificou-se aumento significativo das atividades, no soro lácteo, da GGT no G4 e G6 em relação ao G1 e G3, respectivamente. Os resultados permitem concluir que a mastite subclínica influenciou em todos os parâmetros avaliados. Assim sendo, os parâmetros estudados podem ser classificados como potenciais biomarcadores para a mastite subclínica. No entanto, ao avaliar estes parâmetros, deve-se levar em consideração as fases de lactação, visto que esta influenciou nas variáveis estudadas.

**Palavras-chave:** inflamação; leite; sanidade; lactação



## **INFLUENCE OF LACTATION AND SUBCLINICAL MASTITIS ON TOTAL PROTEIN AND ALBUMIN CONCENTRATIONS AND GGT ACTIVITIES IN THE MILK WHEY OF DAIRY COWS**

The aim of the study was to determine the influence of subclinical mastitis in the composition of the milk whey, taking into account the variations of these components resulting from the physiological changes that occur during lactation. Milk samples from healthy mammary quarters (negative CMT and microbiological culture) of dairy cows in the 1st (G1, n=70), 2nd (G2, n=37) and 3rd (G3, n=147) trimester of lactation, and from mammary quarters with subclinical mastitis (positive CMT and microbiological culture) of dairy cows in the 1st (G4, n=28), 2nd (G5, n=21) and 3rd (G6, n=113) trimester of lactation were collected and evaluated. The concentrations of total proteins (TP) and albumin, as well as gamma glutamyl transferase (GGT) activities, were analyzed, in the milk whey, using a semi-automatic spectrophotometer, at the Laboratory of Clinical Analysis of the Veterinary Hospital of UEM (Umuarama Campus). The parameters were submitted to ANOVA and Tukey's test ( $p < 0.05$ ). The mean $\pm$ SD of the analyzed variables were determined: 1) TP (g/dL) - G1:  $1.20 \pm 0.20$ ; G2:  $1.30 \pm 0.27$ ; G3:  $1.50 \pm 0.30$ ; G4:  $1.60 \pm 0.86$ ; G5:  $1.60 \pm 0.58$ ; G6:  $1.70 \pm 0.62$ ; 2) Albumin (g/dL) - G1:  $0.07 \pm 0.03$ ; G2:  $0.09 \pm 0.03$ ; G3:  $0.10 \pm 0.08$ ; G4:  $0.13 \pm 0.11$ ; G5:  $0.14 \pm 0.07$ ; G6:  $0.17 \pm 0.22$ ; 3) GGT (U/L) - G1:  $2518 \pm 991$ ; G2:  $2895 \pm 1725$ ; G3:  $3151 \pm 1297$ ; G4:  $3436 \pm 2925$ ; G5:  $3337 \pm 1563$ ; G6:  $3810 \pm 1095$ . There was a significant influence of lactation, with higher concentrations of TP and albumin in G2 in relation to G1



and in G3 in relation to G1 and G2. It was also observed higher activities, in the milk whey, of GGT in G3 in relation to G1. There was a significant influence of subclinical mastitis, with higher concentrations of TP and albumin in G4, G5 and G6 compared to G1, G2 and G3, respectively. Furthermore, there was a significant increase in the activities, in the milk whey, of GGT in G4 and G6 in relation to G1 and G3, respectively. The results allow us to conclude that subclinical mastitis influenced all parameters evaluated. Therefore, the parameters studied can be classified as potential biomarkers for subclinical mastitis. However, when evaluating these parameters, lactation must be taken into account, since it influenced the variables studied.

**Keywords:** inflammation; milk; sanity; lactation



## **INFLUÊNCIA DAS FASES DE LACTAÇÃO E DA MASTITE SUBCLÍNICA NAS CONCENTRAÇÕES DE ELETRÓLITOS E MINERAIS NO SORO LÁCTEO DE VACAS LEITEIRAS**

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O objetivo do estudo foi determinar as alterações causadas pela mastite subclínica na composição do soro lácteo, levando-se em consideração as variações destes componentes decorrentes das alterações fisiológicas que ocorrem durante a lactação. Foram coletadas e avaliadas amostras de leite de quartos mamários sadios (CMT e microbiologia negativos) de vacas no 1º (G1, n=70), 2º (G2, n=37) e 3º (G3, n=147) trimestre de lactação, e de quartos mamários com mastite subclínica (CMT e microbiologia positivos) de vacas no 1º (G4, n=28), 2º (G5, n=21) e 3º (G6, n=113) trimestre de lactação. Foram analisados, no soro lácteo, as concentrações de cloretos (Cl), mediante leitura em espectrofotômetro semi-automático, e as concentrações de sódio (Na), potássio (K) e cálcio iônico (Ca<sup>i</sup>), utilizando analisador de eletrólitos, no Laboratório de Análises Clínicas do HV da UEM (Campus de Umuarama). Os parâmetros foram submetidos à ANOVA e ao teste de Tukey ( $p < 0,05$ ). Foi



possível determinar a média $\pm$ DP das variáveis analisadas: 1) Cl (mEq/L)- G1: 197 $\pm$ 34; G2: 173 $\pm$ 27; G3: 182 $\pm$ 23; G4: 209 $\pm$ 36; G5: 200 $\pm$ 21; G6: 205 $\pm$ 28; 2) Na (mEq/L)- G1: 165 $\pm$ 33; G2: 141 $\pm$ 27; G3: 160 $\pm$ 16; G4: 187 $\pm$ 38; G5: 177 $\pm$ 22; G6: 182 $\pm$ 32; 3) K (mEq/L)- G1: 34,0 $\pm$ 4,4; G2: 38,0 $\pm$ 3,4; G3: 35,0 $\pm$ 6,0; G4: 35,0 $\pm$ 7,3; G5: 32,0 $\pm$ 6,7; G6: 28,0 $\pm$ 8,9; 4) Cai (mEq/L)- G1: 3,70 $\pm$ 0,71; G2: 3,10 $\pm$ 0,72; G3: 3,10 $\pm$ 0,85; G4: 3,20 $\pm$ 0,92; G5: 2,80 $\pm$ 0,85; G6: 2,50 $\pm$ 0,81. Observou-se influência significativa das fases de lactação, com maiores concentrações de Cl e Cai no G1 em relação ao G2 e G3; menores concentrações de Na no G2 em relação ao G1 e G3; menores concentrações de K no G1 em relação ao G2. Verificou-se influência significativa da mastite subclínica, com maiores concentrações de Na no G4, G5 e G6 em relação ao G1, G2 e G3, respectivamente; aumento das concentrações de Cl no G5 e G6 em relação ao G2 e G3, respectivamente; diminuição das concentrações de K no G5 e G6 em relação ao G2 e G3, respectivamente; diminuição das concentrações de Cai no G4 e G6 em relação ao G1 e G3, respectivamente. Os resultados permitem concluir que as fases de lactação e a mastite subclínica influenciaram em todos os parâmetros avaliados.

**Palavras-chave:** sanidade; bioquímico; inflamação; leite





## INFLUENCE OF LACTATION AND SUBCLINICAL MASTITIS ON ELECTROLYTE AND MINERAL CONCENTRATIONS IN THE MILK WHEY OF DAIRY COW

The aim was to determine the influence of subclinical mastitis in the composition of the milk whey, taking into account the variations of these components resulting from the physiological changes that occur during lactation. Milk samples from healthy mammary quarters (negative CMT and microbiology) of dairy cows in the 1st (G1, n=70), 2nd (G2, n=37) and 3rd (G3, n=147) trimester of lactation, and from mammary quarters with subclinical mastitis (positive CMT and microbiology) of dairy cows in the 1st (G4, n=28), 2nd (G5, n=21) and 3rd (G6, n=113) trimester of lactation were collected and evaluated. The concentrations of chlorides (Cl) were analyzed by semi-automatic spectrophotometer, and the concentrations of sodium (Na), potassium (K) and ionic calcium (iCa), by an electrolyte analyzer, at the Laboratory of Clinical Analysis of the Veterinary Hospital of UEM (Umuarama Campus). The parameters were submitted to ANOVA and Tukey's test ( $p < 0.05$ ). The mean $\pm$ SD of the analyzed variables were determined: 1) Cl (mEq/L) - G1: 197 $\pm$ 34; G2: 173 $\pm$ 27; G3: 182 $\pm$ 23; G4: 209 $\pm$ 36; G5: 200 $\pm$ 21; G6: 205 $\pm$ 28; 2) Na (mEq/L) - G1: 165 $\pm$ 33; G2: 141 $\pm$ 27; G3: 160 $\pm$ 16; G4: 187 $\pm$ 38; G5: 177 $\pm$ 22; G6: 182 $\pm$ 32; 3) K (mEq/L) - G1: 34.0 $\pm$ 4.4; G2: 38.0 $\pm$ 3.4; G3: 35.0 $\pm$ 6.0; G4: 35.0 $\pm$ 7.3; G5: 32.0 $\pm$ 6.7; G6: 28.0 $\pm$ 8.9; 4) iCa (mEq/L) - G1: 3.70 $\pm$ 0.71; G2: 3.10 $\pm$ 0.72; G3: 3.10 $\pm$ 0.85; G4: 3.20 $\pm$ 0.92; G5: 2.80 $\pm$ 0.85; G6: 2.50 $\pm$ 0.81. There was a significant influence of lactation, with higher concentrations of Cl and iCa in G1 compared to G2 and G3; lower concentrations of Na in G2 in relation to G1



and G3; lower concentrations of K in G1 compared to G2. There was a significant influence of subclinical mastitis, with higher concentrations of Na in G4, G5 and G6 in relation to G1, G2 and G3, respectively; increase in Cl concentrations in G5 and G6 in relation to G2 and G3, respectively; decrease in K concentrations in G5 and G6 in relation to G2 and G3, respectively; decreased concentrations of iCa in G4 and G6 in relation to G1 and G3, respectively. The results allow us to conclude that the phases of lactation and subclinical mastitis influenced all parameters evaluated.

**Keywords:** health; biochemist; inflammation; milk



## **INFLUÊNCIA DO ESTRESSE TÉRMICO SOBRE PARÂMETROS COMPORTAMENTAIS E DE SANIDADE EM BOVINOS LEITEIROS**

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Estresse térmico é caracterizado por alterações fisiológicas e comportamentais devido ao calor ambiental excessivo. O mesmo ocorre quando a temperatura externa ultrapassa a zona de conforto térmico do bovino entre 5°C e 25 °C graus. As principais alterações que ocorrem quando o bovino está sob efeito do calor excessivo são: diminuição de ingestão de matéria seca, aumento da frequência respiratória para equilibrar sua temperatura corporal e redução do tempo em descanso. Nestes casos, os animais se mantêm em estação para facilitar a troca de calor com o ambiente. Diante disto, o objetivo do presente estudo foi comparar a influência de diferentes épocas do ano sobre o tempo de ruminação, ofegação tempo de atividade e tempo de ócio de vacas leiteiras. A coleta de dados foi realizada em uma fazenda comercial por meio de informações retrospectivas de 18 vacas que foram avaliadas pelo colar de monitoramento (Cowmed®). Alguns parâmetros como tempo de ruminação, ofegação, tempo de atividade e tempo de ócio foram comparados entre os meses de verão (Fevereiro e março) e de inverno (Junho e julho). Para obtenção dos resultados foram realizados os testes estatísticos WILCOXON e teste de Spearman pelo software Graphpad prism



9.0<sup>®</sup> ( $p < 0,05$ ). Quando avaliados os dados, a taxa de ofegação foi maior durante o verão quando comparado com o inverno. Esse cenário indica que o animal se apresenta em estresse térmico resultando tentativa de manter a homeotermia do organismo por meio do aumento da frequência respiratória e ofegação. Ao comparar a taxa de atividade, esta foi maior no período de inverno do que no verão ( $p = 0,0001$ ). A taxa de ócio foi maior no período do verão ( $p = 0,0001$ ) quando comparada com o período de inverno. A taxa de ruminação e os demais parâmetros avaliados apresentaram correlação positiva quando comparados com a taxa de temperatura e umidade ( $p < 0,0084$ ,  $r = 0,1$ ). A partir desses dados pode-se observar que durante o verão os animais apresentaram menor tempo de ruminação, maior taxa de ofegação, maior tempo de atividade e de ócio, demonstrando uma influência térmica na produtividade destes animais.

**Palavras-chaves:** Estresse calórico, conforto, sanidade



## **INFLUENCE OF HEAT STRESS ON BEHAVIORAL AND HEALTH PARAMETERS IN DAIRY CATTLE**

Heat stress is characterized by physiological and behavioral changes due to excessive environmental heat. It occurs when the external temperature exceeds the thermal comfort zone of the bovine between 5°C and 25°C degrees. The main changes that occur when cattle are under the effect of excessive heat are: decreased dry matter intake, increased respiratory rate to balance their body temperature and reduced resting time. In these cases, the animals are kept in station to facilitate heat exchange with the environment. In view of this, the objective of the present study was to compare the influence of different seasons of the year on rumination time, panting activity time, and idle time of dairy cows. Data collection was performed in a commercial farm by means of retrospective information from 18 cows that were evaluated by the monitoring collar (Cowmed®). Some parameters as rumination time, panting, activity time and idle time were compared between summer (February and March) and winter (June and July) months. To obtain the results, the WILCOXON statistical test and Spearman test were performed using Graphpad prism 9.0® software ( $p < 0.05$ ). When the data were evaluated, the rate of panting was higher during the summer when compared to winter. This scenario indicates that the animal is under thermal stress resulting in an attempt to maintain body homeothermia by increasing respiratory rate and panting. When comparing the activity rate, it was higher in winter than in summer ( $p = 0.0001$ ). The idle rate was higher in the summer period ( $p = 0.0001$ ) when compared to the winter period. The rumination rate and the



other parameters evaluated showed a positive correlation when compared to the temperature and humidity rate ( $p < 0.0084$ ,  $r = 0.1$ ). From these data it can be observed that during the summer the animals had less cud chewing time, higher panting rate, increased activity time and idle time, showing a thermal influence on the productivity of these animals.

**Keywords:** caloric stress, comfort, health



## INFLUÊNCIA DO FORNECIMENTO DE COLOSTRO FRESCO E CONGELADO NA MICROBIOTA INTESTINAL E RESPOSTA INFLAMATÓRIA EM BEZERROS NEONATOS

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O objetivo deste estudo foi avaliar a capacidade das células do colostro em modular a colonização microbiana intestinal, a atividade da resposta inflamatória e sua influência no desenvolvimento da diarreia em bezerros. Vinte bezerros foram distribuídos em dois grupos, que receberam colostro: COL+ (n = 10) integral fresco; COL- (n = 10) congelado em *pool*, sem células viáveis. Todas as avaliações foram feitas antes da ingestão do colostro (D0), no dia seguinte (D2) e semanalmente no 7º (D7), 14º (D14), 21º (D21) e 28º (D28) dia de idade. A diarreia foi avaliada por escore fecal e o estado inflamatório sistêmico foi avaliado pela combinação de temperatura, anemia, nível sérico total de ferro e haptoglobina, além da necessidade de tratamento antimicrobiano sistêmico. Foram selecionadas bactérias relacionadas à saúde intestinal (*Lactobacillus* spp.



e *Bifidobacterium* spp.) e com potencial patogênico ao intestino (*Escherichia coli* e *Clostridium perfringens*). A diarreia foi observada pela primeira vez em D2 e atingiu o pico nesses bezerros entre D7 e D21, e sua incidência foi semelhante em ambos os grupos. O número de bactérias indicadoras presentes na população fecal foi estimado usando qPCR. No entanto, os bezerros COL- apresentaram sinais mais frequentes de resposta inflamatória sistêmica, incluindo febre no D7 ( $P = 0,011$ ); níveis de haptoglobina em D7 e D14, e níveis mais baixos de ferro em D7 e D14. A anemia foi detectada mais frequentemente nos bezerros COL- no D21 ( $P = 0,043$ ) e D28 ( $P = 0,016$ ). Os bezerros COL- tiveram chance 1,66 maior de ter haptoglobina elevada e chance 1,8 maior de precisar de tratamento com antimicrobianos do que COL+. Menor número de cópias de DNA de *Clostridium perfringens* foi detectado em bezerros COL+ em D2 ( $P = 0,088$ ) e D7 ( $P = 0,040$ ). Da mesma forma, baixo número de cópias de DNA foi observado para *Escherichia coli* e *Lactobacillus* spp. ( $P = 0,012$ ) nas amostras fecais de bezerros COL+ no D7. Esse estudo indicou que as células colostrais maternas tiveram efeito modulador na colonização por organismos, que são frequentemente patogênicos e ajudam a controlar o desenvolvimento de diarreia e suas consequências inflamatórias em bezerros.

**Palavras-chave:** bezerros leiteiros; células colostrais; *Clostridium perfringens*; colonização bacteriana; *Escherichia coli*





## INFLUENCE OF FRESH AND FROZEN COLOSTRUM FEEDING ON GUT MICROBIOTA AND INFLAMMATORY RESPONSE IN NEONATAL CALVES

The aim of this study was to evaluate the capacity of cells from colostrum to modulate the intestinal microbial colonization, the activity of the inflammatory response, and for their influence on the development of diarrheal disease in calves. Twenty calves were distributed into two groups: COL+ ( $n = 10$ ) receiving fresh whole colostrum; COL- ( $n = 10$ ) receiving pooled frozen colostrum, containing no viable cells. All assessments were made before colostrum intake (D0), the next day (D2), and weekly on the 7<sup>th</sup> (D7), 14<sup>th</sup> (D14), 21<sup>st</sup> (D21) and 28<sup>th</sup> (D28) day of age. Diarrhea was assessed using a fecal score, and the systemic inflammatory status was assessed using a combination of temperature, anemia, total serum iron level, total haptoglobin concentration and the need for systemic antimicrobial treatment. For this study, bacteria related to intestinal health (*Lactobacillus* spp. and *Bifidobacterium* spp.), and bacteria with pathogenic potential for the intestine (*Escherichia coli* and *Clostridium perfringens*) were selected. Diarrhea was first observed on D2 and peaked in these calves between D7 and D21. Diarrhea incidence was similar in both groups. The number of indicator bacteria present in the fecal population was estimated using qPCR. However, COL- calves presented more frequent signs of systemic inflammatory response including, fever at D7 ( $P = 0.011$ ); indicator haptoglobin levels on D7 and D14, and lower levels of iron on D7, D14. Anemia was detected



more often in the COL- calves on D21 ( $P = 0.043$ ) and D28 ( $P = 0.016$ ). COL- calves had a 1.66 greater chance of having elevated haptoglobin and a 1.8 greater chance of needing treatment with antimicrobials than COL+. A lower number of DNA copies of *Clostridium perfringens* were detected in COL+ calves on D2 ( $P = 0.088$ ) and D7 ( $P = 0.040$ ). Similarly, a low number of DNA copies was observed for *Escherichia coli* and *Lactobacillus* spp. ( $P = 0.012$ ) in the fecal samples of COL+ calves on D7. This study indicated that maternal colostrum cells had a modulating effect on colonization by organisms that are often pathogens and help manage the development of diarrhea and its inflammatory consequences in calves.

**Keywords:** bacterial colonization; colostrum cells; *Clostridium perfringens*; dairy calves; *Escherichia coli*



## **INTERAÇÃO ENTRE PERCEPÇÃO DE RISCO E PRÁTICAS DE BIOSSEGURIDADE PARA O BVDV E BOHV-1 EM REBANHOS LEITEIROS DA REGIÃO DOS CAMPOS GERAIS DO PARANÁ**

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Biosseguridade é o conjunto de práticas introduzidas em um sistema produtivo a fim de prevenir e/ou controlar a entrada, disseminação e saída de agentes biológicos. O objetivo principal desta pesquisa foi levantar os principais riscos biológicos em rebanhos da região dos Campos Gerais do Paraná. O estudo ocorreu através da parceria entre a Frísia Cooperativa Agroindustrial e USP, e os custos subsidiados pelo Programa Mais Leite Saudável (MAPA). Foram elaborados e aplicados formulários de análise de risco biológico em 69 sistemas de produção de leite majoritariamente compostos por fêmeas Holandesas (91,30%; 63/69), com média de 183 vacas de lactação, produção individual de 32 litros/dia, CCS de 221.000 células/mL, e produção total de 460.745 de leite por dia. As respostas foram pontuadas e as propriedades categorizadas segundo o grau de percepção de risco sendo este alto, médio ou baixo (37,68%,26/69;



31,88%,22/69; 30,43%,21/69, respectivamente). A análise de múltipla correspondência (MCA) foi realizada para explorar associações entre a percepção de risco dos produtores e práticas de manejo envolvendo os agentes virais. Ela nos traz resultados em forma de dimensões, responsáveis pela variabilidade do desfecho estudado. Assim, a maioria dos produtores (88,41%, 61/69) possuíam conhecimento sobre as viroses, e já haviam buscado mais informações para proteger seus rebanhos dessas doenças (61/69; 50/69, respectivamente). Apesar disso, 50,72% dos produtores consideravam suas fazendas desprotegidas a esses vírus (35/69). Em relação à saúde dos animais, o alto grau de percepção de risco correspondeu com a maior necessidade de auxiliar os partos das fêmeas ( $d_1=52,97\%$ ;  $d_2=25,05\%$ ). Já a baixa percepção de risco, estava associada a ausência de medidas para contenção desses agentes como: procedimentos de limpeza e desinfecção dos locais após abortos, envio do material para análise e separação das fêmeas que abortaram ( $d_1=30,58\%$ ;  $d_2=23,61\%$ ). Também houve correspondência entre a prática de compra de animais gestantes anterior ou atual com o baixo grau de percepção de risco ( $d_1=40,66\%$ ;  $d_2=33,33\%$ ). Esses achados demonstram que o grau de percepção de risco está associado às características atitudinais do produtor em relação à implementação de práticas de biossegurança direcionadas à prevenção do BVDV e BoHV-1.

**Palavras-chaves:** análise de risco; programas de controle; análise de correspondência



## **INTERACTION BETWEEN RISK PERCEPTION AND BIOSECURITY PRACTICES FOR BVDV AND BOHV-1 IN DAIRY HERDS IN THE CAMPOS GERAIS REGION OF PARANÁ**

Biosecurity is a series of practices introduced in a production system in order to prevent and/or control the entry, dissemination and exit of biological agents. The main objective of this research was to survey the main biological risks in cattle in the Campos Gerais region of Paraná. The study took place through a partnership between Frísia Cooperativa Agroindustrial and USP, and the More Healthy Milk Program (MAPA) subsidized the costs. Analysis of biological risk forms were elaborated and applied to 69 dairy production systems, mostly composed of Holstein cows (91.30%; 63/69), an average of 183 lactating cows and individual production of 32 liters/day, SCC of 221.000 cells/mL, and total production of 460.745 milk per day. The answers were scored and the properties categorized according to the degree of risk perception considered high, medium or low (37.68%, 26/69; 31.88%, 22/69; 30.43%, 21/69, respectively). Multiple correspondence analysis (MCA) was performed to explore associations between farmers' perception of risk and management practices involving viral agents. It brings us results in the form of dimensions, responsible for the variability of the outcome studied. Thus, most producers (88.41%, 61/69) had knowledge about viruses, and had already searched for more information to protect their herds from these diseases (61/69; 50/69, respectively). Despite this, 50.72% of the producers considered their farms unprotected against these viruses (35/69).



Regarding animal health, the high degree of risk perception corresponded with greater need of assistance to calving ( $d1=52.97\%$ ;  $d2=25.05\%$ ). Low perception of risk was associated with the absence of actions to contain these agents, such as cleaning and disinfection procedures of areas after abortions, delivery of material for analysis and separation of females that aborted ( $d1=30.58\%$ ;  $d2=23.61\%$ ). There was also a correspondence between previous or current practice of purchasing pregnant animals and low degree of risk perception ( $d1=40.66$ ;  $d2=33.33$ ). These findings demonstrate that risk perception degree is associated with producer's attitudinal characteristics regarding the implementation of biosecurity practices directed to the prevention of BVDV and BoHV-1.

**Keywords:** risk analysis; control programs; correspondence analysis



## LEPTOSPIROSE GENITAL BOVINA: ALTA VARIEDADE DE ESTIRPES CAUSANDO A SÍNDROME NO ESTADO DO RIO DE JANEIRO

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A Leptospirose Genital Bovina (BGL) é uma síndrome que leva a falhas reprodutivas em bovinos, apresentando curso crônico e subclínico. Por ter sido descrita recentemente, a maioria dos dados da BGL foram baseados em estudos envolvendo animais assintomáticos em abatedouro, e portanto, não havia conhecimento do histórico reprodutivo destes. Portanto, o objetivo deste estudo foi comparar geneticamente sequências de *Leptospira* spp. obtidas de vacas vivas e com histórico de falhas reprodutivas, com as sequências obtidas dos bovinos assintomáticos de abatedouro. Para isto, foram coletados fragmentos uterinos (FU), muco cervicovaginal (MCV) e urina de 48 vacas destinadas ao descarte devido a falhas reprodutivas. Os animais pertenciam a quatro rebanhos do estado do Rio de Janeiro. As amostras foram submetidas a PCR de *lipL32* (gene exclusivo de leptospiros patogênicas), e posteriormente, a nested-PCR do gene *secY* para o sequenciamento nucleotídico. Dos animais coletados, 25



vacas foram PCR-positivas, e destas, 21 sequências genéticas foram obtidas. As sequências foram classificadas como *Leptospira interrogans* (16 animais), *L. noguchii* (2), *L. santarosai* (1) e *L. borgpetersenii* (1). A análise filogenética foi realizada para avaliar a similaridade genética das sequências encontradas neste estudo com as outras sequências de bovinos já depositadas. Das vacas que foram positivas, 73% apresentaram infertilidade crônica, 50% apresentaram repetição de estro e 11,5% tiveram abortamentos. Embora o estudo tenha demonstrado uma alta diversidade de espécies de leptospiros, mesmo em animais de uma mesma região, *L. interrogans* foi mais frequente. A análise filogenética também demonstrou que as sequências encontradas neste estudo possuem alta similaridade com outras sequências de FU, MCV e folículo ovariano de animais assintomáticos de estudos anteriores também conduzidos no estado do Rio de Janeiro. Portanto, além demonstrar uma alta variedade de estirpes de *Leptospira* spp. causando a BGL no estado do Rio de Janeiro, a análise molecular demonstrou que os animais assintomáticos dos estudos anteriores possuíam as mesmas estirpes de leptospira que os animais vivos com doença reprodutiva conhecida. Este estudo também ressaltou os sinais crônicos e silenciosos como a infertilidade crônica e repetição de estro como principais manifestações da BGL, sendo os abortamentos menos frequentes.

**Palavras-chave:** leptospirose; análise molecular; portadores genitais; infertilidade; abortamento





## BOVINE GENITAL LEPTOSPIROSIS: A HIGH DIVERSITY OF STRAINS CAUSING THE SYNDROME IN RIO DE JANEIRO STATE

Bovine genital leptospirosis (BGL) is a syndrome that leads to reproductive failures in cattle, presenting chronic and silent manifestations. Because of its recent description, the main data of BGL was based on studies involving asymptomatic bovines at slaughterhouses, consequently, there was no reproductive history data of those animals. Therefore, the aim of this study was to perform a genetic comparison of *Leptospira* spp. sequences obtained from live cows with a history of reproductive failures with the sequences obtained from asymptomatic cows of those previous studies. For this reason, it was collected uterine fragments (UF), cervicovaginal mucus (CVM), and urine from 48 live cows destined to culling due to reproductive failures. The animals belonged to four herds in Rio de Janeiro state. The samples were submitted to PCR of the *lipL32* gene (exclusive of pathogenic leptospires), and later to a nested-PCR of the *secY* gene for nucleotide sequencing. Of the studied animals, 25 cows were positive for *lipL32*-PCR, and 21 genetic sequences were obtained. The sequences were classified as *Leptospira interrogans* (16 animals), *L. noguchii* (2), *L. santarosai* (1) and *L. borgpetersenii* (1). The phylogenetic analysis was performed in order to evaluate the genetic similarity of the sequences found herein and the sequences of bovines that were previously deposited. Of the positive cows, 73% showed chronic infertility, 50% had estrus repetition and 11.5% had abortion episodes. Although this study had demonstrated a high diversity of leptospires,



even from animals of the same region, *L. interrogans* was the more frequent strain. The phylogenetic analysis also showed that the sequences found herein had high similarity with other sequences of UF, CVM, and ovarian follicle from previous studies involving asymptomatic cows at slaughterhouses also from Rio de Janeiro state. Therefore, besides this study reinforces that there is a diversity of leptospires causing BGL in Rio de Janeiro state, the molecular analysis showed that the asymptomatic animals of previous studies had the same strains as the live animals with known reproductive failures. This study also highlighted those chronic and silent signs as the major manifestation of BGL and abortions as less frequent.

**Keywords:** leptospirosis, molecular analysis, genital carriers, infertility, abortions



## LESÃO DE NERVO FIBULAR E DEFORMIDADE FLEXURAL EM BOVINO – RELATO DE CASO

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A lesão do nervo fibular é comum em quadros de decúbito prolongado, devido sua posição anatômica superficial, causando consideráveis perdas econômicas. O nervo fornece motricidade aos músculos flexores do jarrete e extensores do dígito e sensibilidade ao dorso do metatarso, boleto e dígitos. A sintomatologia da injúria está intimamente relacionada com sua função e o prognóstico é favorável para casos unilaterais agudos diagnosticado prontamente. Foi atendido na região de Choró, Ceará, uma fêmea bovina, sem padrão racial definido, 7 anos, 400 kg, aptidão leiteira, com o histórico de “febre do leite”, seguido por 5 dias de decúbito. Na ocasião, foi elaborado um suporte para mantê-la em estação e em 15 dias o animal começou a locomover-se com dificuldade, sendo integrada à rotina do rebanho. Após 12 meses, o proprietário solicitou atendimento veterinário. No exame clínico, observou-se que a fêmea



não realizava a extensão dos dígitos do membro posterior esquerdo, apresentava pouca sensibilidade na região dorsal do membro, o casco demonstrava desgaste irregular da pinça, crescimento exacerbado dos talões, feridas na região dorsal da coroa e deformidade flexural dos tendões flexores digitais (TFD) superficial (TFDS) e profundo (TFDP). Fechou-se o diagnóstico de paralisia neuromuscular pós-parto por lesão do nervo fibular com deformidade flexural adquirida. Conforme a literatura, realizou-se tratamento cirúrgico por meio da tenotomia do TFDS e TFDP. No pós-operatório instituiu-se terapia antimicrobiana, anti-inflamatória e antitetânica. Como terapia local estabeleceu-se a aplicação de bandagem e tala moldada para o membro do animal. A troca da bandagem ocorria a cada 72 horas para limpeza da ferida. Cuidados adicionais envolveram casqueamento corretivo e restrição dos movimentos. Na evolução pós-cirúrgica ocorreu adequada cicatrização da ferida e a vaca passou caminhar sem claudicar. O tratamento indicado para lesões em nervos periféricos, especialmente em casos agudos, envolve acondicionar o animal em cama macia, trocar a posição do decúbito, fisioterapia e terapia sistêmica com anti-inflamatórios. Contudo, no caso relatado o atendimento veterinário foi solicitado após um ano da lesão e com sequelas motoras já instaladas, restando apenas a opção do tratamento cirúrgico. Apesar da cronicidade, o tratamento utilizado foi efetivo e proporcionou a melhora clínica do animal.

**Palavras-chave:** hipocalcemia; decúbito; injúria; nervo periférico



## **FIBULAR NERVE INJURY AND FLEXURAL DEFORMITY IN BOVINE – CASE REPORT**

The peroneal nerve injury is common in cases of prolonged decubitus, due to its superficial anatomical position, causing considerable economic losses. The nerve provides motricity to the flexor muscles of the hock and extensors of the digit and sensitivity to the dorsum of the metatarsal, fetlock and digits. The symptomatology of the injury is closely related to its function and the prognosis is favorable for acute unilateral cases diagnosed promptly. A bovine female, without defined racial pattern, 7 years old, 400 kg, dairy aptitude, with a history of “milk fever”, followed by 5 days of recumbency, was treated in the region of Choró, Ceará. On that occasion, a support was created to keep it in season and in 15 days the animal began to move around with difficulty, being integrated into the herd's routine. After 12 months, the owner requested veterinary care. In the clinical examination, it was observed that the female did not perform the extension of the digits of the left hind limb, had little sensitivity in the dorsal region of the limb, the hoof showed irregular wear of the forceps, exacerbated growth of the heels, wounds in the dorsal region of the crown and flexural deformity of the superficial (TFDS) and deep (TFDP) digital flexor tendons. The diagnosis of postpartum neuromuscular paralysis due to peroneal nerve injury with acquired flexural deformity was made. According to the literature, surgical treatment was performed by means of tenotomy of the TFDS and TFPD. In the postoperative period, antimicrobial, anti-inflammatory and anti-tetanic therapy was instituted.



As a local therapy, the application of a bandage and molded splint to the animal's limb was established. The bandage was changed every 72 hours to clean the wound. Additional care involved corrective trimming and restriction of movements. In the post-surgical evolution, adequate wound healing occurred and the cow walked without lameness. The treatment indicated for peripheral nerve injuries, especially in acute cases, involves placing the animal in a soft bed, changing the position of the decubitus, physiotherapy and systemic therapy with anti-inflammatory drugs. However, in the case reported, veterinary care was requested one year after the injury and with motor sequelae already installed, leaving only the option of surgical treatment. Despite the chronicity, the treatment used was effective and provided the clinical improvement of the animal.

**Keywords:** hypocalcemia; recumbency; injury; peripheral nerve



## LEVANTAMENTO DE DIAGNÓSTICO DIRETO EM AMOSTRAS DE BOVINOS COM SINAIS RESPIRATÓRIOS REALIZADO NO INSTITUTO BIOLÓGICO (2020-2022)

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Os laboratórios da Sanidade Animal (CPDSA) do Instituto Biológico processaram 74 amostras de tecidos bovinos com queixa de sinais respiratórios (informações do remetente) no período de janeiro 2020 a Setembro 2022 para detecção direta de agentes infecciosos bacterianos e/ou virais. Em 31 casos (42%) além dos sinais respiratórios, foram relatados também sinais gastrintestinais e em 4 (5,4%) sinais neurológicos e 3 casos com alteração nos três sistemas (4,05%). A maior ocorrência concentrou-se nas estações outono/inverno (64%), sendo 25 animais <6 meses (35%), 2 com 6-12 meses de idade (3%), 20 > 1 ano (28%) e 25 (35%) sem informação. Dentre os Estados atendidos, a maioria era proveniente do Estado SP (75,68%) e 17,5% MG, sendo 81,67% propriedades de aptidão leiteira. Das 47 amostras processadas apenas para cultivo bacteriológico verificou-se associação de patógenos em 4 (8,5%) entre eles: *Mannheimia haemolytica*, *Salmonella* Dublin, *Salmonella* Enteritidis e *Clostridium perfringens* A (CPA). Em alguns casos também detectou-se isoladamente CPA (1), *S. Dublin*



(2) e *M. haemolytica* (3). Das 5 amostras analisadas para micobactéria, uma (20%) foi positiva para *Mycobacterium bovis* e uma apresentou contaminação. Treze amostras (17,57%) foram analisadas exclusivamente para presença viral onde detectou-se BVDV em 2 (15,38%). Somente 9 amostras (12,16%) foram analisadas concomitantemente para bactérias e vírus, e em 6 (66,7%) houve algum resultado significativo sendo uma amostra positiva para BoHV-1, uma para CPA, uma para BVDV, uma para *S. Dublin* e CPA, e duas (22,22%) resultaram em associação bacteriana e viral: *P. multocida* e BRSV; e *M. haemolytica*, *S. Dublin*, CPA e BVD. As amostras de conveniência apresentadas aqui foram oriundas de óbito em criações sem acompanhamento veterinário hospitalar, cuja suspeita e definição das análises foram determinadas pelo solicitante. Mesmo o diagnóstico bacteriológico e virológico tendo sido feito paralelamente em baixa quantidade de amostras, a diversidade de agentes encontrados tanto em perfil isolado quanto associado, destaca a importância do diagnóstico laboratorial amplo. Além da complexidade para avaliação clínica do animal enfermo a campo, a análise laboratorial pontual pode mascarar a real situação da propriedade – muitas vezes multicausal- levando a tomada de medidas de controle incompletas e, portanto, ineficazes.

**Palavras-chave:** gastrintestinal, bactérias, vírus, micobactéria, co-infecção





## **DIRECT DIAGNOSIS FROM BOVINE SAMPLES WITH RESPIRATORY SIGNALS PROCESSED BY INSTITUTO BIOLÓGICO SURVEY (2020-2022)**

Instituto Biológico- Animal Health Center (CPDSA) laboratories processed 74 bovine tissue samples with respiratory signals (according to sender information) from January 2020 to September 2022 for direct diagnosis of bacteria and virus pathogens. In 31 events (42%), beyond respiratory signs, it was also reported gastrointestinal and in 4 (5.4%) neurological signs. In three cases (4.05%) all three systems were affected. Highest occurrence concentrated during autumn/winter seasons (64%), and out of 47 events with age information, 25 <6 months (35%), two 6-12 months old (3%) and 20 (28%) > 1 year old animals were affected. Among the attended States, the majority was from SP (75.68%) and 17.5% from MG, besides 81.67% were classified as dairy properties. Out of 47 samples submitted only to bacteriological culture, in 4 (8.5%) was observed different profiles of these pathogens co-infection: *Mannheimia haemolytica*, *Salmonella* Dublin, *Salmonella* Enteritidis and *Clostridium perfringens* A(CPA). In a few cases there was single pathogen detection like CPA (1), *S. Dublin* (2) and *M. haemolytica* (3). From the 5 samples submitted to mycobacteria analysis one (20%) was positive and typified as *Mycobacterium bovis*. Thirteen samples (17.57%) were processed solely for viral detection and BVDV was detected in two (15.38%). Only 9 (12.16%) samples were concurrently analyzed for bacteria and virus presence, and in 6 (12.16%) was found some significant result with one



positive sample for BoHV-1, one for CPA, one for BVDV, another one for both S. Dublin and CPA, and two samples (22.22%) showed bacteria and virus association: *P. multocida* and BRSV; *M. haemolytica*, S. Dublin, CPA and BVDV. Convenience samples presented in this study were originated from death animals in cattle raising with no veterinary hospital support, where suspicion and analysis definition were settled by the sender. Even though the complete analysis was performed in a few samples, the diversity of pathogens reported as single or in association highlights the importance of large laboratory diagnosis. Besides the complexity for clinically evaluating a field diseased animal, punctual laboratory diagnosis can mask the real property situation – often multicausal – leading to incomplete control measures and, therefore, ineffective.

**Keywords:** gastrointestinal, bacteria, virus, mycobacteria, co-infection



## **MASTITE EM PROPRIEDADE O papel do anticorpo anti-*E. coli* do S DE BOVINO LEITEIRO EM ASSENTAMENTO RURAL NO NOROESTE DE SP**

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Mastite bovina é enfermidade que acomete a glândula mamária de vacas acarretando grandes prejuízos econômicos, devido à diminuição da produção leiteira e com o gasto com uso de medicamentos. O presente trabalho foi realizado em um assentamento, em Promissão, Estado de São Paulo, onde existem pequenos produtores com baixa qualidade do leite. Foram coletadas 189 amostras de leites de 56 animais que apresentaram aumento da celularidade através do teste CMT (California Mastitis Test) para realizar exames bacteriológicos e antibiograma. Os leites foram coletados em recipientes estéreis e acondicionados em isopor com gelo até o processamento. Para o exame bacteriológico, 10 µL das amostras do leite foram semeados em ágar sangue de carneiro 5%, incubados por 48 horas a 37°C. Foram isoladas 155 colônias, sendo: 132 *Staphylococcus* spp. (85,16%), 13 *Bacillus* sp (8,32%), 5 *Streptococcus* sp (3,22%), 2 fungos (1,29%), 1 *Corynebacterium bovis* (0,64%) 1 *Escherichia coli* (0,64%) e um Bacilo Gram negativo não fermentador (GNNF) (0,64%). Do total de amostras, 37 (23,87%) não apresentaram crescimento



bacteriano. Para o gênero *Staphylococcus* spp., mais prevalente no estudo, foram utilizados os seguintes discos de antibióticos: azitromicina ( $\mu\text{g}$ ), cefalotina (30  $\mu\text{g}$ ), ceftiofur ( $\mu\text{g}$ ), cloranfenicol (30  $\mu\text{g}$ ), tetraciclina (30  $\mu\text{g}$ ), gentamicina (10  $\mu\text{g}$ ), penicilina (10 UI), eritromicina ( $\mu\text{g}$ ), amoxicilina (25 $\mu\text{g}$ ) e sulfazotrim (25  $\mu\text{g}$ ). Das 132 colônias, 72 (54,5%) foram sensíveis a todos os antibióticos; 22 (16,6%) foram resistentes à amoxicilina, 21 (15,9%) foram resistentes à amoxicilina, cefalotina e eritromicina, concomitantemente, 9 (6,81%) à azitromicina, eritromicina e amoxicilina e 8 (6,06%) à eritromicina, amoxicilina, penicilina e sulfazotrim. As propriedades do assentamento utilizaram como tratamento ceftiofur, antibiótico que não promoveu resistência frente às estirpes de *Staphylococcus* encontradas no presente trabalho, além de ser de aplicação em dose única e pouca eliminação no leite, o que não acarreta o descarte do leite dos animais tratados e, conseqüentemente, prejuízo ao produtor. No presente trabalho verificamos alta resistência bacteriana frente aos antibióticos testados, provavelmente ao devido a uso indiscriminado, uso incorreto de dosagem e tempo de administração, o que aumenta os casos de resistência bacteriana. Esse cenário propicia a perpetuação dos casos de mastite nas propriedades leiteiras em nosso país.

**Palavras chave:** *Staphylococcus* , leite, multidrogas resistência



## MASTITIS IN DAIRY CATTLE FARMS IN A SETTLEMENT IN THE NORTHWEST OF SP

Bovine mastitis is a disease of mammary glands from dairy cows resulting in economic damages due to milk discard and decreased production associated with the costs of treatment. This study aimed to assess the situation occurring with small producers from Promissao municipality, Sao Paulo State, Brazil, reporting problems of milk quality. After sampling of 56 animals positive by California Mastitis Test (CMT) with increased somatic cell counts, 189 milk samples were obtained for bacteriological isolation, characterization and antibiogram profile. Milk samples were appropriately collected into sterile tubes and kept refrigerated until analysis. For bacterial culture, 10  $\mu$ L of each sample was inoculated on blood agar base enriched with 5% sheep blood and plates were incubated for 48 hours at 37°C. A total of 155 growths were observed, specifically: 132 *Staphylococcus* spp. (85.16%), 13 *Bacillus* sp (8.32%), 5 *Streptococcus* sp (3.22%), 2 fungi (1.29%), 1 *Corynebacterium bovis* (0.64%), 1 *Escherichia coli* (0.64%) and 1 non-fermenting Gram-negative bacilli (0.64%). No growth were shown in 37 samples (23.87%). For the most prevalent pathogen, *Staphylococcus* spp., the following antibiotics were tested: azithromycin ( $\mu$ g), cephalothin (30  $\mu$ g), ceftiofur ( $\mu$ g), chloramphenicol (30  $\mu$ g), tetracyclin (30  $\mu$ g), gentamicin (10  $\mu$ g), penicillin (10 UI), erythromycin ( $\mu$ g), amoxicillin (25 $\mu$ g) and sulfazothrim (25  $\mu$ g). All the antibiotics showed good efficacy for 72 of the 132



(54.5%) isolates; 22 (16.6%) specimen were resistant to amoxicillin; 21 (15.9%) showed concomitant resistance to amoxicillin, cephalothin and erythromycin, 9 (6.81%) to azithromycin, erythromycin and amoxicillin and 8 (6.06%) presented multiresistance to erythromycin, amoxicillin, penicillin and sulfazothrim. Treatment with ceftiofur was reported to be used in the studied herds, but we did not observe *Staphylococcus* resistance against this antibiotic, which is administrated in single dose and is poorly released through milk, therefore, diminishing losses and leading to economic advantages for the producers. Nonetheless, a considerable percentagem of antibiotic resistance was detected over the samples analyzed, probably caused by incorrect and extensive drug administration. Proper management and continuous surveillance will be recommended to prevent spreading of acute cases associated with multiresistant bacteria, a very common situation in Brazil.

**Keywords:** *Staphylococcus*, milk, multidrugs resistance



## **MONITORAMENTO E AVALIAÇÃO DO COLOSTRO, TRANSFERÊNCIA DE IMUNIDADE PASSIVA E GANHO DE PESO DE BEZERROS FILHOS DE VACAS TRATADAS COM COMPOSTO HOMEOPÁTICO**

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A homeopatia, que tem por base o preparo de substâncias ultra diluídas e sucussionadas, tornou-se popular na bovinocultura leiteira, por ser uma terapia cujo objetivo é promover a saúde e bem-estar aos tratados, mas que tem a vantagem, frente outras terapias, de não deixar resíduo no leite. O colostro, consiste em uma mistura de secreções lácteas, imunoglobulinas e outras proteínas séricas, de suma importância para os bezerros, já que nesta espécie a transferência de imunidade da mãe para o filho ocorre essencialmente fora do útero, através da ingestão do colostro. O objetivo deste trabalho foi avaliar o colostro de seis vacas prenhe, previamente tratadas com o complexo homeopático Convert H da Real H®, durante 5 meses, e aferir a transferência



de imunidade passiva e ganho de peso dos bezerros nascidos. As avaliações foram realizadas logo após o parto, e o peso dos bezerros foi acompanhado até 60 dias de vida. As vacas foram divididas em grupo tratado (GT) e grupo controle (GC). O GT foi tratado com 15g/cab/dia, sendo que o produto foi incorporado à ração recebida, e os animais do GC, recebiam apenas a ração sem incorporação do medicamento. A avaliação do colostro e do soro sanguíneo dos bezerros foi realizada através de refratômetro de Brix. O colostro foi avaliado logo após a parição, durante a coleta na primeira ordenha. Já o soro sanguíneo, foi obtido através da coleta de sangue da veia jugular dos bezerros recém-nascidos após 24 horas do nascimento. A coleta foi realizada em tubos estéreis contendo gel separador. Os bezerros eram pesados com fita de pesagem ao nascer e aos 30 e 60 dias de vida. Três bezerros nascidos de cada grupo foram avaliados, totalizando 6 animais. Como resultado, os bezerros nascidos das vacas homeopatizadas apresentaram maior peso ao longo dos 60 dias de vida, com relação ao colostro das vacas e valor de proteínas séricas encontrado no sangue dos bezerros, não houve diferença significativa entre os animais. Para próximos trabalhos, sugere-se um ?n? maior de animais para uma avaliação de maior impacto.

**Palavras-chave:** Imunoglobulinas, homeopatia, bem-estar





## **MONITORING AND EVALUATION OF COLOSTRUM, TRANSFER OF PASSIVE IMMUNITY AND WEIGHT GAIN OF CALVES BORN TO COWS TREATED WITH A HOMEOPATHIC COMPOUND**

Homeopathy is based on the preparation of ultra-diluted substances, which has the advantage of being more popular in cattle farming, which aims to promote the health and well-being of treaties, which has the advantage, compared to the purpose of therapies, of not letting residue in milk. Colostrum consists of a mixture of milk secretions and other proteins, immunoglobulins, of paramount importance for calves, since in this species it is an immunological pregnancy from the mother to the child that essentially occurs outside the uterus, through the ingestion of colostrum. The objective of this work was evaluated before being tested with the homeo complex Convert H from Real H®, for 5 months, and the download of the immunity test is passive and zero weight gain born. Estimates were presented shortly after calving, and the weight of the calves was followed up to 60 days of life. The cows were strongholds in the treated group (TG) and control group (CG). The GT was treated 15g/b/day, with the product being incorporated into the delivery upon receipt, and the animals from the CG received only food without incorporation of the drug. The evaluation of colostrum and blood serum of calves was performed using a Brix refractometer. Colostrum was evaluated shortly after parturition, during first-order collection. Blood serum was collected by collecting blood from the jugular vein of newborn calves 24 hours after birth. The collection was performed in sterile tubes and separators containing gel. The calves were two with weighing tape at birth and at 30 and 60 days of age. Three calves born



from each group were confirmed, totaling 6 animals. As a result, calves born from homeopathized cows did not present greater weight throughout the 60 days of life, in relation to the colostrum of the cows and the value of serum proteins found in the blood of the calves, a significant difference between the animals. For the next works, a larger  $n$  of animals is suggested for a greater impact assessment.

**Keywords:** Immunoglobulins, homeopathy, welfare



## **NECROSE DO CORDÃO ESPERMÁTICO PÓS-ORQUIECTOMIA EM BEZERRO DA RAÇA NELORE – RELATO DE CASO**

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O presente trabalho relata o caso clínico de um bezerro macho da raça nelore, 270 kg, de 4 meses de idade, que apresentou intercorrências durante o processo cicatricial pós-orquiectomia, oriundo de uma propriedade de criação de bovinos de corte na mesorregião do nordeste paraense, localizada no município de Igarapé-Açú, Pará, Brasil. Após 12 dias do procedimento cirúrgico, observou-se que o animal apresentava apatia, perda de peso, além de reação inflamatória exacerbada e com características de cronicidade no local, sendo observada a presença de necrose do segmento distal do cordão espermático remanescente, o qual se apresentava com aspecto firme, enegrecido e seco. O tratamento consistiu na intervenção cirúrgica com remoção do tecido necrosado. Para



realização do procedimento, o animal foi previamente contido e submetido ao protocolo anestésico com Xilazina 0,3mg/kg intramuscular (IM), Cetamina 1mg/kg, e Diazepam 0,04mg/kg intravenoso (IV), posteriormente o bezerro foi posicionado em decúbito lateral direito. Imediatamente, realizou-se a higienização e antissepsia do local da cirurgia com antisséptico químico, gluconato de clorexidina e álcool etílico 70%. Em seguida, evidenciou-se o cordão espermático e efetuou-se o desbridamento e remoção da área tecidual necrosada, para então fazer a ligadura com fio estéril monofilamentado não absorvível náilon tamanho 1-0, em área viável do cordão espermático. Ademais, implementou-se a antibióticoterapia (Enrofloxacino- 1ml/40Kg) e anti-inflamatório (Cetoprofeno 10%, 1,5ml/50kg) durante 5 dias, por via de administração intramuscular (IM). Nota-se que, apesar desta técnica cirúrgica ser utilizada com frequência, principalmente no manejo de bovinos de corte, é possível que ocorram intercorrências devido ao material de sutura, em alguns casos, agir como fator irritativo permanente, interferindo na resposta inflamatória local, causando transtornos à fisiologia da cicatrização; destarte, a retirada do tecido necrosado associada ao uso de antibacteriano e anti-inflamatório demonstrou efetividade em casos de intercorrências pós-cirúrgicas e ressalta a importância do acompanhamento de colaboradores e do médico veterinário na evolução clínica da cicatrização após a realização do procedimento cirúrgicos.

**Palavras-chave:**bovinocultura de corte, castração, sanidade, bezerro



## POST-ORCHIECTOMY SPERMATIC CORD NECROSIS IN A NELORE CALF - CASE REPORT

The present study reports the clinical case of a 4-month-old male calf of the breed Nelore, 270 kg, that presented complications during the healing process after orchietomy, from a beef cattle breeding property in the northeast region of Pará, located in the municipality of Igarapé-Açú, Pará, Brazil. Twelve days after the surgical procedure, the animal presented apathy, weight loss, and an exacerbated inflammatory reaction with chronic characteristics at the site. Necrosis of the distal segment of the remaining spermatic cord was observed, with a firm, blackened and dry aspect. The treatment consisted in surgical intervention with removal of the necrosed tissue. To perform the procedure, the animal was previously restrained and submitted to anesthetic protocol with intramuscular (IM) Xylazine 0.3 mg/kg, Ketamine 1 mg/kg, and intravenous (IV) Diazepam 0.04 mg/kg, after which the calf was positioned in right lateral decubitus. Immediately, the surgical site was cleaned and antiseptically cleaned with chemical antiseptic, chlorhexidine gluconate and 70% ethyl alcohol. Then, the spermatic cord was evidenced and debridement and removal of the necrotic tissue area were performed, and then the ligature was performed with sterile, non-absorbable nylon monofilament suture size 1-0, in a viable area of the spermatic cord. In addition, antibiotic therapy (Enrofloxacin - 1ml/40Kg) and anti-inflammatory (Cetoprofen 10%, 1.5ml/50kg) were administered intramuscularly (IM) for 5 days. Although this surgical technique is frequently used, especially in the management of beef cattle, it is possible that interurrences occur due to the suture material, in some cases, acting as a permanent irritant, interfering in the



local inflammatory response, causing disturbances to the healing physiology; Therefore, the removal of necrotic tissue associated with the use of antibacterial and anti-inflammatory drugs has shown to be effective in cases of post-surgical complications and highlights the importance of the follow-up of collaborators and the veterinarian in the clinical evolution of healing after the surgical procedure.

**Keywords:** beef cattle, castration, health, calf



## O IMPACTO DOS FATORES MATERNOS SOBRE A VIDA REPRODUTIVA DE NOVILHAS DA RAÇA HOLANDESA

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Os fatores maternos como o número de partos, tipo de parto, prematuridade, peso ao nascer, época de nascimento e estresse térmico materno podem iniciar uma série de eventos em cascata que influenciam o desenvolvimento e saúde das bezerras, conseqüentemente esses fatores afetará a vida reprodutiva. As proles que passaram por algum estresse durante o seu crescimento gestacional provocado pelos fatores maternos nascem fracos e com pouca vitalidade, prejudicando a sucção e ingestão do colostro, aumentando então os riscos de contaminação e doenças, principalmente diarreia e alterações respiratórias. O objetivo desta pesquisa foi avaliar o impacto dos fatores maternos sobre a performance reprodutiva de novilhas Holandesas. Foram selecionadas 226 vacas Holandesas e suas proles. Os fatores maternos avaliados nesta pesquisa foram categorizados da seguinte forma: 1. Partos eutócicos (n=174) e distócicos (n=52); 2. Duração da gestação:  $\leq 169$  dias = parto prematuro (n=39) e  $\geq 170$  dias = parto a termo (n=187); 3. Paridade = primíparas (n=113) e múltíparas (n=113); e Estações do Ano (nascimento): verão (59), outono (79) e inverno (88); 5. Distribuição das bezerras de acordo com o peso ao nascer  $\leq 35$ Kg (n=20), entre 36 a 40Kg (n=196) e entre 41 a 45 kg (n=10). Novilhas nascidas de vacas



múltiplas ( $P=0.05$ ) e nascidas a termo ( $P=0.07$ ) são inseminadas mais cedo. A época do nascimento também pode impactar no desenvolvimento reprodutivo, observando-se que as novilhas nascidas no verão podem apresentar maior risco de perdas gestacionais (28.57%), em relação às novilhas nascidas no outono (11.90%) e inverno (7.04%). Esses valores podem impactar na idade do 1º parto. Novilhas nascidas no inverno pariram mais precocemente (17,83 meses), já as que nasceram no outono (23,04 meses) e primavera (19,01 meses) pariram mais velhas. Os fatores maternos comprometem os mecanismos placentários e adaptação pós-natal de bezerras, diminui a vitalidade, esse fato dificulta a ingestão de colostro devido pouco interesse durante a administração do colostro, tornando-os ainda mais susceptíveis a infecções e contaminações de agentes infecciosos. Assim, nós acreditamos que isso pode ocasionar menor desenvolvimento e resposta imune, comprometendo o processo adaptativo pós-desmame e produtividade futura desses animais

**Palavras-chave:** Reprodução, prematuridade, distócia, estação do ano, peso ao nascer





## IMPACT OF MATERNAL FACTORS OVER REPRODUCTIVE LIFE OF HOLSTEIN HEIFERS

Maternal factors such as number of calvings, type of calving, prematurity, birth weight, time of birth, season of birth and maternal heat stress can initiate a series of cascading events that influence the development and health of calves, consequently these factors will affect its reproductive life. The offspring that underwent some stress during their gestational growth caused by maternal factors are born weak and with little vitality, impairing suction and ingestion of colostrum, thus increasing the risks of contamination and diseases, mainly diarrhea and respiratory disturbances. The objective of this research was to evaluate the impact of maternal factors on the reproductive performance of Holstein heifers. 226 Holstein cows and their offspring were selected. The maternal factors evaluated in this research were categorized as follows: 1. Eutocic (n=174) and dystocic (n=52) births; 2. Duration of pregnancy:  $\leq 169$  days = premature delivery (n=39) and  $\geq 170$  days = full-term delivery (n=187); 3. Number of births = primiparous (n=113) and multiparous (n=113); and seasons of the year (birth): summer (59), autumn (79) and winter (88); 5. Distribution of calves according to birth weight  $\leq 35$  kg (n=20), between 36 to 40 kg (n=196) and between 41 to 45 kg (n=10). Heifers born to multiparous cows ( $P=0.05$ ) and born at term ( $P=0.07$ ) are inseminated earlier. The season of birth can also impact reproductive development, noting that heifers born in summer may have a higher risk of pregnancy loss (28.57%), compared to heifers born in autumn (11.90%)



and winter (7.04%). These values may impact the age of 1<sup>st</sup> calving. Heifers born in winter calved earlier (17.83 months), while those born in autumn (23.04 months) and spring (19.01 months) calved older. Maternal factors compromise placental mechanisms and postnatal adaptation of calves, decrease vitality, this fact makes colostrum ingestion difficult due to little interest during colostrum administration, making them even more susceptible to infections and contamination of infectious agents. Thus, we believe that this can lead to lower development and immune response, compromising post-weaning adaptive process and future productivity of these animals.

**Keywords:** Reproduction, prematurity, dystocia, seasons, birth weight



## O PAPEL DO ANTICORPO ANTI-*E. coli* DO COLOSTRO MATERNO NA COLONIZAÇÃO DO INTESTINO DE BEZERROS LEITEIROS RECÉM-NASCIDOS COM *Escherichia coli* E NO DESENVOLVIMENTO DE DIARREIA CLÍNICA

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*Escherichia coli* (*E. coli*) é uma bactéria comumente transferida da vaca e do ambiente para o bezerro. Para entender o impacto da transferência de IgG, anticorpos específicos de *E. coli* e fatores imunológicos gerais para na microbiota intestinal do bezerro, um estudo foi realizado em bezerras recém-nascidas da raça Holandesa, e acompanhou a colonização do intestino por *E. coli*. Os animais foram submetidos ao “padrão ouro” de manejo do colostro, incluindo a vacinação da mãe contra *E. coli* no pré-parto para aprimorar a produção do colostro. Utilizaram-se 20 vacas da raça Holandesa, com produção de quantidade e qualidade adequada de colostro, e suas bezerras nascidas de partos eutócicos. Realizou-se escore fecal diário para monitoramento de diarreia em cada bezerra. Amostras de sangue e fezes foram coletadas no D1 (antes da ingestão do colostro), depois no D3, D7, D14 e D28 após a colostragem. *E. coli* isoladas de amostras fecais das bezerras foram avaliadas quanto aos genes de virulência e



a quantidade por grama de fezes usando PCR convencional e qPCR, respectivamente. A quantidade total de IgG no plasma e o título de anticorpo de ligação à *E. coli* foram medidos por ELISA. O pico de diarreia foi observado no D12. Tanto a IgG total quanto o anticorpo de ligação aumentaram acentuadamente em D3, e diminuiu com a idade do bezerro. O número total de bactérias (16S rRNA) nas fezes foi semelhante entre D1 e D14, e aumentou durante o período D14 a D28. O número de *E. coli* foi mínimo em D1, aumentou durante o período D3-D7, e diminuiu entre D14-D28. O gene da toxina estável (ST) foi frequentemente identificado na *E. coli* isolada das bezerras, e *E. coli* K99 não foi encontrada. Enquanto o intestino foi colonizado por *E. coli* no início da vida correspondente ao nível máximo de IgG e anticorpos específicos, número decrescente de *E. coli* foi observado à medida que a imunidade passiva diminuiu. Isso sugeriu que a função do anticorpo passivo pode controlar a colonização e estimular a função imune inata que estabilizou o nível de *E. coli* no trato digestório dos bezerros.

**Palavras-chave:** saúde animal, gado leiteiro, diarreia, IgG, transferência de imunidade passiva



## THE ROLE OF ANTI-*E. Coli* ANTIBODY FROM MATERNAL COLOSTRUM ON THE COLONIZATION OF THE GUT OF NEWBORN DAIRY CALVES WITH *Escherichia coli* AND THE DEVELOPMENT OF CLINICAL DIARRHEA

*Escherichia coli* (*E. coli*) is a commonly transferred species of bacteria from the dam and the environment to the calf. To understand the impact of transfer IgG, *E. coli* specific antibody and general immune factors to the calf on the gut microbiota, a study that followed the temporal colonization of the intestine with *E. coli* as correlated with the total quantity of IgG and the antibody recognizing *E. coli* was conducted in newborn Holstein calves. The calves were subjected to the “gold standard” of colostrum management, including of *E. coli* vaccination of the dam prior to delivery to prime colostrum. Twenty Holstein dams, and healthy female offspring were utilized. Each calf was monitored and scored daily for diarrhea. Blood and fecal samples were collected on D1 (before colostrum intake), then D3, D7, D14 and D28 after colostrum feeding. *E. coli* isolated from fecal samples of the calves were assessed for virulence genes and the quantity per gram of feces using conventional PCR and qPCR, respectively. The total quantity of plasma IgG, and the titer of *E. coli* binding antibody were measured by ELISA. The peak in diarrhea in the calves was observed on D12. Both total IgG and binding antibody sharply increased by D3. Total and binding IgG declined with calf age. The total number of bacteria (16S rRNA) in feces was similar from D1 to D14, while from D14 to D28 increased. The number of *E. coli* was minimal on D1, increased during the period D3-D7, and decreased between D14-D28. The stable toxin (ST) gene was frequently identified in the *E. coli* isolated from



the calves, and *E. coli* K99 was not found. While the gut was colonized by *E. coli* early in life corresponding to the peak level of IgG and specific antibody, a decreasing number of *E. coli* were observed as the passive immunity waned. This suggested that the function of the passive antibody may be to control colonization and seed innate immune function that then stabilizes the level of *E. coli* in the digestive tract of calves.

**Keywords:** animal health, dairy cattle, diarrhea, IgG, passive immunity transfer



## **O USO DA DUPLA COLOSTRAGEM AUMENTA A EFICIÊNCIA DA TRANSFERÊNCIA DE IMUNIDADE PASSIVA EM BEZERROS DA RAÇA GIROLANDO CRIADOS NO TRÓPICO ÚMIDO AMAZÔNICO?**

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A colostragem é de fundamental importância para a vida dos bezerros. Quando esta não ocorre de maneira adequada observa-se uma falha na transferência de imunidade passiva (FTIP), que é um fator relevante para a alta taxa de morbimortalidade de bezerros dentro da cadeia produtiva do leite. O objetivo deste trabalho foi avaliar se o uso da segunda colostragem aumenta a eficiência da transferência de imunidade passiva (TIP) em bezerros da raça girolando criados no trópico úmido amazônico. O estudo foi conduzido em uma fazenda localizada no município de Paragominas, Pará. Foram avaliados 80 bezerros (28 Machos e 52 Fêmeas) da raça Girolando, elegidos em delineamento



inteiramente casualizado, e alocados em dois grupos: 1) manejo convencional (n = 40, 1 colostragem) e 2) - dupla colostragem (n = 40), mantidos em bezerreiro tipo tropical. Somente foram incluídos no estudo, bezerros nascidos de partos eutócicos monitorizados, separados de suas mães, tempestivamente, após o nascimento. Os animais eram acompanhados diariamente, desde o nascimento até os 60 dias de idade. Foram colostrados utilizando-se colostro descongelado em banho-maria, à temperatura de 35 a 40°C, grau Brix igual a 25%, ofertados em um volume de 10% do peso corpóreo nas primeiras duas horas pós-nascimento. Para a segunda colostragem, 5% do peso corpóreo em colostro era ofertado ao bezerro em até no máximo 18 horas após o nascimento. Quando o colostro descongelado não atingia esse percentual, era enriquecido com colostro artificial em pó, consoante determinação do fabricante até atingir Brix igual a 25%. Pela determinação do Brix sérico o grupo de animais ao qual foi fornecida a dupla colostragem (10,8<sup>o</sup> Bx) apresentou diferença estatística significativa (P < 0,05), se comparado ao grupo dos animais com manejo convencional (10,0<sup>o</sup>Bx). Em seguida ocorre uma redução (P > 0,05) das 48 horas aos 60 dias de vida mantendo-se ainda dentro dos níveis bons (8,9 - 9,3<sup>o</sup>Bx) a excelentes (≥ 9,4<sup>o</sup>Bx). Desta forma a dupla colostragem demonstra ser uma alternativa viável, pois oferece um incremento significativo na TIP para o sistema de cria em um dos períodos de maior desafio para os bezerros, as primeira 24 horas de idade.

**Palavras-chave:** Brix sérico, manejo, neonato, sanidade, saúde





## **DOES THE USE OF DOUBLE COLOSTRAGE INCREASE THE EFFICIENCY OF PASSIVE IMMUNITY TRANSFER IN GIROLANDO CALVES BREED IN THE MOIST AMAZON TROPIC?**

Colostrum is of fundamental important for calves. When this does not occur properly, there is a failure in the transfer of passive immunity, which is a relevant factor for the high morbidity and mortality rate of calves within the milk production chain. The objective of this work was to evaluate the use of second colostration to increase the efficiency of passive immunity transfer in girolando calves raised in the humid tropics of the Amazon. The study was conducted on a farm located in the municipality of Paragominas, Pará. Eighty calves (28 males and 52 females) of the Girolando breed were evaluated, chosen in a completely randomized design, and allocated into two groups: conventional management group (n = 40, single colostrum) and double colostrum (n = 40), being accommodated in a tropical calf. Only calves born from monitored eutocic deliveries were included in the study. The calves were separated from their mothers, timely, after birth. The animals were monitored daily, from birth to 60 days old, and any change in the animal's health status was identified and the definitive diagnosis established. The animals were colostrated using thawed colostrum in a water bath, at a temperature of 35 to 40°C, Brix degree equal to 25%, offered in a volume of 10% of body weight in the first two hours after birth.



When the thawed colostrum did not reach this percentage, it was enriched with artificial colostrum powder, as determined by the manufacturer, until it reached a Brix equal to 25%. By the determination of serum Brix, the group of animals to which double colostrum was provided (10.8° Bx) showed a statistically significant difference ( $P < 0.05$ ), compared to the group of animals with conventional management (10.0° Bx). Then there is a reduction ( $P > 0.05$ ) from 48 hours to 60 days of life, still remaining within good (8.9 - 9.3°Bx) to excellent levels ( $\geq 9.4^\circ\text{Bx}$ ). In this way, double colostrum proves to be a viable alternative, as it offers a significant increase in IPT for the calf system in one of the most challenging periods for calves, the first 24 hours old.

**Keywords:** Serum brix, management, neonate, sanity, health



## OBSTRUÇÃO DUODENAL POR CORPO ESTRANHO EM BEZERRA GIROLANDO: RELATO DE CASO

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As obstruções intestinais tendo como etiologia intussuscepções, torções e bezoários, são menos frequentes que outras enfermidades gastrointestinais, entretanto, os bezoários também estão diretamente ligados a dieta. Objetiva-se relatar um caso de obstrução duodenal por corpo estranho em bezerra Girolando atendida no Hospital Veterinário da Universidade Federal do Espírito Santo. O animal de quatro meses, proveniente de propriedade leiteira com sistema intensivo de produção, era mantido em piquete de *Brachiaria* spp. com mineralização e água *ad libitum* junto de outros animais da mesma categoria, apresentava histórico de apatia, decúbito esternal, ausência de ruminação e defecação. Clinicamente apresentava apatia, em decúbito esternal, anorexia, desidratação grave e taquicardia, distensão abdominal ventral com timpanismo ruminal e presença de líquido ao balotamento. O hemograma evidenciou hemoconcentração, hiperproteinemia e hiperfibrinogenemia, enquanto o leucograma apresentou leucocitose por neutrofilia com desvio à esquerda leve e regenerativo além de linfocitose. Na bioquímica sérica, a creatinina se mostrou



duas vezes maior que os valores de referência e a proteína total aumentada. A análise do fluido ruminal apresentou comprometimento da microbiota ruminal e elevação do teor de cloreto (104 mEq/L). Previamente a cirurgia, realizou-se descompressão ruminal por sondagem orogástrica e trocaterização. A bezerra foi submetida à laparotomia exploratória do flanco direito, onde constatou-se enterite duodenal focal com obstrução por corpo estranho. Optou-se por realizar enterotomia para retirada do corpo estranho de seis centímetros, leve, acastanhado e formado pela combinação de saco de ração, pelos e material vegetal, sugestivo de pilobezoar. Foi instituído protocolo terapêutico e no décimo dia com a melhora do quadro clínico-patológico o animal recebeu alta clínica. Em bezerros, as doenças digestivas estão comumente associadas a estomatites e diarreias, enquanto obstruções intestinais são pouco relatadas se comparado aos animais adultos. As obstruções por fitobezoares são mais frequentes que por pilobezoares e as causas em bezerros de dois a quatro meses, em fase de aleitamento, ainda não sejam muito bem elucidadas. O diagnóstico de obstrução duodenal por corpo estranho em bezerra Girolando foi confirmado a partir do histórico, fatores epidemiológicos, achados clínico patológicos e cirúrgicos.

**Palavras-chave:** Distúrbios digestivos, enteropatias, enterotomia, laparotomia exploratória, trato gastrointestinal



## **DUODENAL OBSTRUCTION BY FOREIGN BODY IN GIROLANDO CALF: CASE REPORT**

Intestinal obstructions having as etiology intussusceptions, torsion and bezoar are less frequent than other gastrointestinal diseases, however, bezoar are also directly linked to diet. The objective is to report a case of duodenal obstruction by a foreign body in a Girolando calf treated at the Veterinary Hospital of the Federal University of Espírito Santo. The four-month-old animal, from a dairy farm with an intensive production system, was kept in a *Brachiaria* spp pasture, with mineralization and water ad libitum along with other animals of the same category, had a history of apathy, in esternal decubitus, absence of rumination and defecation. Clinically, he presented apathy, in esternal decubitus, anorexia, severe dehydration and tachycardia, ventral abdominal distension with ruminal bloat and presence of fluid on ballooning. The blood count showed hemoconcentration, hyperproteinemia and hyperfibrinogenemia, while the leukogram showed leukocytosis due to neutrophilia with mild and regenerative left shift in addition to lymphocytosis. In serum biochemistry, creatinine was twice as high as the reference values and total protein was increased. The analysis of rumen fluid showed impairment of the rumen microbiota and increased chloride content (104 mEq/L). Prior to surgery, ruminal decompression was performed by orogastric tube and trocar. The calf underwent exploratory laparotomy of the right flank, where focal duodenal enteritis with obstruction by a foreign body was observed. It was decided to perform an enterotomy to remove the foreign body measuring six centimeters, light, brownish and formed by the combination of a



bag of food, hair and plant material, suggestive of pilobezoar. A therapeutic protocol was instituted and on the tenth day, with the improvement of the clinicopathological condition, the animal was discharged. In calves, digestive diseases are commonly associated with stomatitis and diarrhea, while intestinal obstructions are rarely reported when compared to adult animals. Obstructions by phytobezoars are more frequent than by pilobezoars and the causes in calves aged two to four months, in the lactation phase, are still not very well elucidated. The diagnosis of duodenal obstruction by a foreign body in a Girolando calf was confirmed based on the history, epidemiological factors, clinical, pathological and surgical findings.

**Keywords:** Digestive disorders, enteropathies, enterotomy, exploratory laparotomy, gastrointestinal tract



## OCORRÊNCIA DE INFECÇÃO POR *Trypanossoma vivax* EM BOVINO NA REGIÃO DE PIRASSUNUNGA-SP – RELATO DE CASO

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Para o sistema de produção animal o surgimento de doenças e o seu controle são importantes entraves no crescimento. O controle das doenças causadas por protozoários representa enorme desafio, devido à alta morbidade. As tripanossomoses são enfermidades causadas por parasitas hemoflagelados, que afetam seres humanos e animais, para a espécie bovina o protozoário *Trypanosoma vivax* é considerado um agente mais patogênico. O presente trabalho relata o caso de uma fêmea bovina, adulta, da raça holandesa, atendida em 27 de abril de 2016 pelo Serviço de Buiatria e Clínica de Pequenos Ruminantes da Unidade Didática Clínica Hospitalar (UDCH) da Universidade de São Paulo, em Pirassununga-SP. O proprietário relatou que dois dias anteriores o animal havia gerado um bezerro fraco, que foi a óbito 24 horas após o nascimento, e havia diminuído a ingestão de alimento e a produção de leite. Ao



exame clínico, foi observado retenção dos anexos fetais, edema e laceração vulvar e presença de ectoparasitas. No hemograma, apresentava anemia normocítica normocrômica e leve leucopenia. Iniciou-se o tratamento de suporte e dos sinais clínicos apresentados e o animal ficou em internamento, o qual era monitorado através do exame clínico duas vezes ao dia. Após uma semana o quadro reprodutivo foi estabelecido satisfatoriamente, porém, foi observado febre recorrente (3 dias seguidos) e debilidade. Coletou-se amostras de sangue para novo hemograma e realizado o esfregaço sanguíneo da ponta de orelha, no qual foi identificado *Trypanosoma* spp. O animal foi tratado com Diaceturato de 4-, 4- Diazoaminodibenzamidina (Pirentel®), na dose única de 7 mg/kg, IM (repetido após 15 dias), além de tratamento de suporte. Não houve resposta satisfatória do animal ao tratamento, apresentando debilidade progressiva, com comprometimento respiratório, picos de febre, fraqueza muscular e decúbito, vindo a óbito um mês após detecção do agente no esfregaço sanguíneo. Nas amostras de sangue coletadas durante o período de internamento, foram realizadas extração de DNA e PCR para identificação de genes específicos para identificação do *T. vivax* e ELISA de IgG anti-*T. vivax*. Os resultados obtidos pelos testes moleculares confirmaram se tratar do protozoário hemoflagelado *Trypanosoma vivax*.

**Palavras-chave:** Vaca leiteira. Tripanossomose. PCR. ELISA





## **OCCURRENCE OF *Trypanossoma vivax* INFECTION IN CATTLE IN THE REGION OF PIRASSUNUNGA-SP - CASE REPORT**

For the animal production system, the emergence of diseases and their control are important obstacles in growth. The control of diseases caused by protozoa represents a huge challenge, due to the high morbidity. Trypanosomoses are diseases caused by hemoflagellate parasites, which affect humans and animals, para the bovine species the protozoan *Trypanosoma vivax* is considered a more pathogenic agent. The present work reports the case of a female bovina, adult, of the Dutch race, attended on April 27, 2016, by the Buiatry and Clinic of Small Ruminants of the Hospital Clinical Didactic (UDCH) unit of the University of São Paulo, in Pirassununga-SP. The owner reported that two days earlier the animal had generated a weak calf, which died 24 hours after birth, and had decreased food intake and milk production. On clinical examination, fetal attachment retention, vulvar edema and laceration and presence of ectoparasitas were observed. On the blood count, he presented normochromic normocytic anemia and mild leukopenia. The supportive treatment and clinical signs presented were started and the animal was hospitalized, which was monitored through clinical examination twice a day. After one week the reproductive condition was satisfactorily established, however, recurrent fever (3 days in a row) and weakness was observed. Blood samples were collected for a new blood count and blood smear from the ear tip was performed, in which *Trypanosoma* spp. The animal was treated with Diaceturate of 4-, 4- Diazoaminodibenzamidine (Pirentel®), at a single dose of 7 mg/kg, IM (repeated after 15 days), in addition to



supportive treatment. There was no satisfactory response of the animal to the treatment, presenting progressive weakness, with respiratory impairment, fever peaks, muscle weakness and decubitus, dying one month after detection of the agent in the blood smear. In the blood samples collected during the hospitalization period, dna extraction and PCR were performed to identify specific genes to identify *T. vivax* and ELISA of IgG anti-*T. vivax*. The results obtained by molecular tests confirmed that it is the hemoflagellate protozoan *Trypanosoma vivax*.

**Keywords:** Cows. Trypanosomoses. PCR. ELISA



## PATÓGENOS PERIODONTAIS PUTATIVOS NA MICROBIOTA SUBGENGIVAL DA PERIODONTITE BOVINA

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A periodontite bovina é uma enfermidade infecciosa, multifatorial e polimicrobiana que culmina com a destruição dos tecidos de sustentação dos dentes e eventual perda dentária, e causa mudanças cumulativas que ocorrem durante toda a vida, afetando o bem-estar e a produtividade animal. A presença de periodontopatógenos é necessária para o desenvolvimento desta condição em seres humanos e outras espécies animais, e esses agentes patogênicos provavelmente desempenham um papel importante na periodontite bovina. O presente estudo teve por objetivo avaliar pela reação em cadeia da polimerase (PCR) e com iniciadores específicos a presença de 35 espécies de periodontopatógenos na bolsa periodontal de bovinos com lesões de profundidade maior que 5 milímetros (n=26) e no sulco gengival de animais considerados clinicamente sadios (n=25). A análise de prevalência e risco foi realizada por meio do teste t de Student e correlação de Spearman. Nos 26 animais com lesões periodontais, os micro-organismos mais prevalentes foram *Fusobacterium nucleatum* (96,2%), *Fusobacterium necrophorum* (80,7%),



*Actinomyces naeslundii* (80,7%), *Porphyromonas endodontalis* (80,7%), *Prevotella melaninogenica* (73,1%) e *Treponema amylovorum* (73,1%). Já nos 25 bovinos sem lesões periodontais, *Fusobacterium nucleatum* (84%), *Eikenella corrodens* (72%), *Treponema amylovorum* (72%), *Treponema maltophilum* (72%) e *Fusobacterium necrophorum* (68%) foram os micro-organismos mais identificados. *Aggregatibacter actinomycetemcomitans*, *Actinomyces viscosus*, *Campylobacter curvus*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Enterobacteriaceae*, *Enterococcus faecalis*, *Porphyromonas gingivalis*, *Porphyromonas gulae*, *Prevotella tanneriae*, *Treponema medium*, *Treponema socranskii* e *Treponema vincentii* não foram detectados em nenhuma das amostras pesquisadas. Os resultados da análise estatística indicaram que a ocorrência de *Actinomyces naeslundii*, *Enterococcus faecium*, *Porphyromonas asaccharolytica*, *Porphyromonas endodontalis*, *Prevotella buccae*, *Prevotella intermedia*, *Prevotella melaninogenica*, *Prevotella nigrescens*, *Prevotella oralis*, *Treponema denticola* e *Treponema pectinovorum* está associada com a periodontite bovina. Assim, os resultados do presente estudo contribuem para a elucidação da etiopatogenia da periodontite bovina.

**Palavras-chave:** doença periodontal, bovinos, periodontopatógenos, PCR



## PUTATIVE PERIODONTAL PATHOGENS IN THE SUBGINGIVAL MICROBIOTA OF BOVINE PERIODONTITIS

Bovine periodontitis is an infectious, multifactorial and polymicrobial disease that culminates in the destruction of the supporting tissues of the teeth and eventual tooth loss, and causes cumulative changes that occur throughout life, affecting animal welfare and productivity. The presence of periodontopathogens is necessary for the development of this condition in humans and other animal species, and these pathogens probably play an important role in bovine periodontitis. The present study aimed to evaluate, by polymerase chain reaction (PCR) and with specific primers, the presence of 35 species of periodontopathogens in the periodontal pocket of cattle with lesions deeper than 5 millimeters (n=26) and in the gingival sulcus of animals considered clinically healthy (n=25). Prevalence and risk analysis was performed using Student's t test and Spearman's correlation. In the 26 animals with periodontal lesions, the most prevalent microorganisms were *Fusobacterium nucleatum* (96.2%), *Fusobacterium necrophorum* (80.7%), *Actinomyces naeslundii* (80.7%), *Porphyromonas endodontalis* (80.7%), *Prevotella melaninogenica* (73.1%) and *Treponema amylovorum* (73.1%). In the 25 cattle without periodontal lesions, *Fusobacterium nucleatum* (84%), *Eikenella corrodens* (72%), *Treponema amylovorum* (72%), *Treponema maltophilum* (72%) and *Fusobacterium necrophorum* (68%) were the most identified microorganisms. *Aggregatibacter actinomycetemcomitans*, *Actinomyces viscosus*, *Campylobacter curvus*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Enterobacteriaceae*,



*Enterococcus faecalis*, *Porphyromonas gingivalis*, *Porphyromonas gulae*, *Prevotella tanneriae*, *Treponema medium*, *Treponema socranskii* and *Treponema vincentii* were not detected in any of the samples. The results of the statistical analysis indicated that the occurrence of *Actinomyces naeslundii*, *Enterococcus faecium*, *Porphyromonas asaccharolytica*, *Porphyromonas endodontalis*, *Prevotella buccae*, *Prevotella intermedia*, *Prevotella melaninogenica*, *Prevotella nigrescens*, *Prevotella oralis*, *Treponema denticola* and *Treponema pectinovorum* was associated with bovine periodontitis. Thus, the results of the present study contribute to the elucidation of the etiopathogenesis of bovine periodontitis.

**Keywords:** periodontal disease, cattle, periodontopathogens, PCR



## PERFIL DE SUSCEPTIBILIDADE DE MICRORGANISMOS ASSOCIADOS À MASTITE CLÍNICA EM PROPRIEDADE RURAL DE GOIÂNIA, GOIÁS

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Mastites são inflamações da glândula mamária que possuem grande impacto econômico na produção leiteira. É dividida nas formas subclínica e clínica, esta última caracterizada por alterações macroscópicas no leite. Os microorganismos causadores podem ser patógenos contagiosos (como *Staphylococcus aureus*) ou ambientais (*Escherichia coli* e *Streptococcus* sp.). A terapia antimicrobiana é importante para controle da doença, porém seu uso indiscriminado contribui para a resistência bacteriana. O presente estudo teve como objetivo avaliar a sensibilidade antimicrobiana de microorganismos isolados do leite de vacas com mastite clínica do rebanho da Fazenda Escola da Universidade Federal de Goiás (UFG). Assim, realizou-se o teste da caneca de fundo escuro para a identificação dos animais doentes. Cada amostra de leite foi submetida a cultura bacteriana, com leitura das colônias em 24, 48 e 72 horas, e realização de testes bioquímicos para identificação do agente. Após, realizou-se a verificação da suscetibilidade



antimicrobiana. Foram excluídos animais com mastite clínica já em tratamento. Dos 58 animais em lactação, 13 apresentaram mastite clínica e foram incluídos no estudo. Dentre as 9 amostras que apresentaram crescimento, os agentes mais prevalentes foram *Streptococcus* sp (30,76%), *Escherichia coli* (30,76%) e *Staphylococcus* sp (15,38%). Das 4 cepas isoladas de *E. coli*, todas apresentaram resistência ou resultado intermediário para fluoroquinolonas, ceftiofur e doxiciclina e 75% à tetraciclina. Resistência antimicrobiana à amoxicilina, enrofloxacino, tetraciclina, azitromicina e ceftriaxone foi identificada em 75% das cepas isoladas de *Streptococcus* sp. e em 50% das cepas de *Staphylococcus* sp. resistência à tetraciclina. Os resultados do presente estudo evidenciam a ocorrência de patógenos multirresistentes na propriedade avaliada. Assim, é necessária a utilização de forma racional dos antimicrobianos através do monitoramento constante dos agentes associados à mastite bovina e a determinação do seu perfil de sensibilidade.

**Palavras-chave:** mastite clínica, antimicrobianos, resistência, sensibilidade





## **SUSCEPTIBILITY PROFILE OF MICROORGANISMS ASSOCIATED WITH CLINICAL MASTITIS IN A RURAL PROPERTY IN GOIÂNIA, GOIÁS**

Mastitis is inflammation of the mammary gland that has a great economic impact on milk production. It is divided into subclinical and clinical forms, the latter characterized by macroscopic changes in milk. The causative microorganisms can be contagious pathogens (such as *Staphylococcus aureus*) or environmental (*Escherichia coli* and *Streptococcus* sp.) pathogens. Antimicrobial therapy is important for disease control, but its indiscriminate use contributes to bacterial resistance. The present study aimed to evaluate the antimicrobial susceptibility of microorganisms isolated from the milk of cows with clinical mastitis from the herd of Fazenda Escola, Universidade Federal de Goiás (UFG). Thus, the dark-bottomed mug test was performed to identify sick animals. Each milk sample was submitted to bacterial culture, with colony reading at 24, 48 and 72 hours, and biochemical tests were performed to identify the agent. Afterwards, the verification of antimicrobial susceptibility was carried out. Animals with clinical mastitis already undergoing treatment were excluded. Of the 58 lactating animals, 13 had clinical mastitis and were included in the study. Among the 9 samples that showed growth, the most prevalent agents were *Streptococcus* sp (30.76%), *Escherichia coli* (30.76%) and *Staphylococcus* sp (15.38%). Of the 4 strains isolated from *E. coli*, all showed resistance or an intermediate result to fluoroquinolones, ceftiofur and doxycycline and 75% to tetracycline. Antimicrobial



resistance to amoxicillin, enrofloxacin, tetracycline, azithromycin and ceftriaxone was identified in 75% of the isolated strains of *Streptococcus* sp. and in 50% of *Staphylococcus* sp. tetracycline resistance. The results of the present study show the occurrence of multidrug-resistant pathogens in the evaluated property. Thus, the rational use of antimicrobials is necessary through constant monitoring of agents associated with bovine mastitis and the determination of their sensitivity profile.

**Keywords:** clinical mastitis, antimicrobials, resistance, sensitivity



## **PERFIL DO REBANHO LEITEIRO NO SUDOESTE DO PARANÁ E A SUA RELAÇÃO COM O EXAME BACTERIOLÓGICO DO LEITE**

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A região Sudoeste do Paraná possui uma bacia leiteira caracterizada por uma produção ascendente, mas as características produtivas dos rebanhos ainda são desconhecidas. Com o intuito de conhecer estes aspectos, o objetivo do presente estudo foi conhecer as características do rebanho, produção leiteira, práticas de biossegurança e qualidade do leite de propriedades localizadas no Sudoeste do Paraná. As informações foram obtidas a partir da aplicação de questionário e análises laboratoriais. Ao todo, foram entrevistados noventa e quatro produtores e realizada três coletas de amostras de leite do tanque de refrigeração com intervalo de trinta dias cada, totalizando duzentas e oitenta e duas amostras. Estas foram coletadas de forma asséptica, acondicionadas em caixa isotérmica e encaminhadas para o Laboratório de Microbiologia da



Universidade Federal da Fronteira Sul para realização do exame bacteriológico. Os resultados obtidos no questionário e nas análises laboratoriais foram tabulados em Tabela Dinâmica e realizada estatística descritiva. A partir dos resultados, foi possível observar que 53% das propriedades possuem até 15 animais no plantel, 47,9% dos produtores utilizavam ordenha mecânica balde ao pé, 57% tem uma produção diária de até 150 litros e cerca de 60% relatam ter rebanho fechado. Com relação ao exame bacteriológico 62% das propriedades apresentaram presença de enterobactérias no leite, 77% *Staphylococcus* spp., e 48% *Streptococcus agalactiae*. Conclui-se que a atividade leiteira na região tem predomínio de pequenos e médios produtores, com número reduzido de animais nas propriedades. Torna-se evidente também que as propriedades têm deficiências de manejo e higiene, os quais repercutem na baixa qualidade bacteriológica do leite entregue ao laticínio, necessitando de adoção de medidas públicas que visem a orientação aos produtores para a melhoria da qualidade do leite produzida na região do Sudoeste do Paraná.

**Palavras-chaves:** qualidade do leite, microbiologia, vacas leiteiras, mastite, *Staphylococcus* spp.



## PROFILE OF DAIRY CATTLE IN THE SOUTHWEST OF PARANÁ AND ITS RELATIONSHIP WITH THE BACTERIOLOGICAL EXAMINATION OF MILK

The Southwest region of Paraná it is an important dairy region but the characteristics of herds on production, and management is still unknown. To understand these aspects, the objective of the present study was to survey those herds for topics such as herd demography, milk production, biosecurity practices, and milk quality. The information was obtained from in-person application of a questionnaire and laboratory analysis of bulk tank milk samples. In total, ninety-four producers were interviewed, and three milk samples were collected from their bulk tank at intervals of thirty days each, totaling two hundred and eighty-two samples. Milk samples were collected aseptically, packed in an isothermal box, and sent to the Microbiology Laboratory of the Universidade Federal da Fronteira Sul for microbiology analysis. The results obtained from the questionnaire and laboratory tests were tabulated in a pivot table, and descriptive statistics were performed. Based on the results, it was possible to observe that 53% of the properties have up to 15 animals, 48% of the producers used manual milking, 57% have a daily production of up to 150 liters, and about 60% reported having a closed herd. Results from the analysis of milk samples indicated that 77% of the properties showed the presence of *Staphylococcus* spp., 62% enterobacteria, and 48% *Streptococcus agalactiae*. The conclusion of the study



showed that dairy farming in the region is predominantly carried out by small-sized producers, with a deficiency in management, and consequent low milk quality, The adoption of measures to increase education to improve better management and milk quality produced in the Southwestern region of Paraná is evident and necessary.

**Keywords:** milk quality, microbiology, dairy cows, mastitis, *Staphylococcus* spp



## PERITONITE SECUNDÁRIA À RUMINOTOMIA – RELATO DE CASO

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A peritonite é a inflamação do peritônio, que pode ocorrer por causas infecciosas ou não infecciosas. Em ruminantes, geralmente são secundárias à úlcera de abomaso, reticulites traumáticas, perfuração da cavidade abdominal, contaminação cirúrgica e inflamação do trato urogenital. Tem relevância em vacas leiteiras no pós-parto devido a maior ocorrência de cirurgias abdominais, úlcera de abomaso e infecções uterinas. O objetivo desse resumo é relatar o caso de uma vaca com peritonite devido à deiscência de pontos decorrente de uma ruminotomia. A paciente bovina, fêmea, raça holandesa, com cerca de 9 anos, foi atendida pelo serviço externo realizado pela equipe da CBPR-USP (Clínica de Bovinos e Pequenos Ruminantes – Universidade de São Paulo). O animal estava apático, com anorexia, pouca ingestão hídrica, febre, dor abdominal ao urinar e liberação de secreção mucosanguinolenta na região da vulva. Foi encaminhada e internada no Hospital Veterinário (HOVET-USP) e estava com baixo escore de condição corporal (1,25), febre (39,7°C), desidratação (10-12%), atonia ruminal, diarreia líquida e corrimento vaginal. O



hemograma apresentava leucocitose (50.000  $\mu$ L), por neutrofilia (47.500  $\mu$ L); e no exame bioquímico do sangue, apresentava hipoproteinemia (4,73 mg/dL), hipoalbuminemia (1,6 mg/dL), e aumento de bilirrubina total. A ultrassonografia mostrou focos hiper ecogênicos em fígado; lojas de fibrina com líquido e líquido heterogêneo livre em cavidade abdominal, além de áreas de consolidação pulmonar. Foi realizada laparotomia exploratória, constatando que havia deiscência de pontos devido a ruminotomia realizada anteriormente, com extravasamento de líquido ruminal com fibrina e aderência de forma difusa na cavidade abdominal, compartimentalizado em diversas lojas, onde destaca-se grande quantidade de gás sob pressão. Animal tinha histórico anterior ao atendimento prestado pela equipe CBPR-USP de reticulite por corpo estranho, aborto posterior a ruminotomia e metrite. Conclui-se que a peritonite pode ser secundária a causas cirúrgicas, com prognóstico geralmente desfavorável, reiterando a importância da técnica cirúrgica adequada.

**Palavras chave:** Bovina, reticulite, deiscência





## PERITONITIS SECONDARY TO RUMINOTOMY - CASE REPORT

Peritonitis is inflammation of the peritoneum, which may occur from infectious or non-infectious causes. In ruminants, it is usually secondary to abomasal ulcer, traumatic reticulitis, perforation of the abdominal cavity, surgical contamination and inflammation of the urogenital tract. It has relevance in postpartum dairy cows due to the higher occurrence of abdominal surgeries, abomasal ulcer and uterine infections. The aim of this abstract is to report the case of a cow with peritonitis due to dehiscence of stitches following a ruminotomy. The patient was a female, Dutch breed, about 9 years old, and was seen by the external service performed by the CBPR-USP (Clinic of Cattle and Small Ruminants - University of São Paulo) team. The animal was apathetic, with anorexia, little water intake, fever, abdominal pain when urinating and release of mucosanguinous secretion in the vulva region. She was referred and admitted to the Veterinary Hospital (HOVET-USP) with a low body condition score (1.25), fever (39.7°C), dehydration (10-12%), rumen atony, liquid diarrhea and vaginal discharge. The hemogram showed leukocytosis (50,000  $\mu\text{L}$ ), by neutrophilia (47,500  $\mu\text{L}$ ); and blood biochemical examination showed hypoproteinemia (4.73 mg/dL), hypoalbuminemia (1.6 mg/dL), and increased total bilirubin. Ultrasonography showed hyperechogenic foci in liver; fibrin stores with fluid and free heterogeneous fluid in abdominal cavity, and areas of lung consolidation. Exploratory laparotomy was performed, and it was found that there was dehiscence of stitches due to a previous ruminotomy, with extravasation of ruminal fluid with fibrin and diffuse adherence in the abdominal cavity,



compartmentalized in several stores, where a large amount of gas under pressure can be seen. The animal had a previous history of foreign body reticulitis, abortion after ruminotomy and metritis. We conclude that peritonitis may be secondary to surgical causes, with generally unfavorable prognosis, reiterating the importance of proper surgical technique.

**Keywords:** Cattle, reticulitis, dehiscence



## REDUÇÃO DO NÚMERO DE APLICAÇÕES DE ACARICIDAS EM BOVINOS POR MEIO DO TRATAMENTO SELETIVO

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Usualmente os pecuaristas utilizam a aplicação de acaricidas em todo o rebanho desde a primavera até o outono com intervalo de aplicação de 21 a 28 dias para o controle do carrapato bovino (*Rhipicephalus microplus*). Isso porque a infestação por carrapatos em bovinos provoca perdas produtivas e eleva os custos de produção. Este estudo parte da hipótese que o tratamento seletivo, no qual a intervenção medicamentosa por acaricidas é realizada apenas nos bovinos com a presença de carrapatos, reduz o número de doses aplicadas. O objetivo foi avaliar o número de aplicações de acaricidas em novilhos submetidos ao tratamento seletivo. O estudo foi realizado na Fazenda Experimental da Universidade Federal do Paraná, em Pinhais, Brasil. Anualmente foram avaliados 36 bovinos da raça Angus, de julho a abril dos anos de 2017, 2018, 2019, 2020 e 2021, totalizando 144 bovinos. A infestação por carrapatos foi estimada quinzenalmente por meio da observação dos animais e contagem de



teleóginas presentes nos bovinos. O animal com a contagem superior a 40 carrapatos foi tratado com acaricida transcutâneo. O número de aplicações foi 74% maior no ano 2019/20. No ano de 2019/20, 6% e 3% dos animais receberam 5 e 6 aplicações, respectivamente. Na média dos anos de avaliação os animais receberam 1,8 doses de acaricidas e 13% dos animais não receberam acaricida. Em relação ao modelo convencional de tratamento, os animais submetidos ao tratamento seletivo tiveram uma economia de acaricidas na ordem de 76%, 79%, 70% e 72%, nos anos de 2017/18, 2018/19, 2019/20 e 2020/21, respectivamente. Desta forma o tratamento seletivo mostra-se uma ferramenta eficiente na redução do número de doses de acaricidas aplicados anualmente com potencial na redução da contaminação ambiental e da pressão de seleção na geração de populações resistentes.

**Palavras-chave:** controle parasitário, carrapato bovino, ectoparasito



## REDUCTION OF THE NUMBER OF ACARICIDES APPLICATIONS IN CATTLE THROUGH SELECTIVE TREATMENT

Usually, ranchers use the application of acaricides throughout the herd from spring to autumn with an application interval of 21 to 28 days to control the bovine tick (*Rhipicephalus microplus*). That's because tick infestation in cattle causes productive losses and increases production costs. This study starts from the hypothesis that selective treatment, whereupon drug intervention by acaricides is performed only in cattle with the presence of ticks, reduces the number of doses applied. The objective was to evaluate the number of acaricide applications in heifers submitted to selective treatment. The study was carried out at the Experimental Farm of the Federal University of Paraná, in Pinhais, Brazil. Annually, 36 Angus cattle were evaluated, from July to April of the years 2017, 2018, 2019, 2020 and 2021, totaling 144 cattle. Tick infestation was estimated fortnightly by observing the animals and counting teleogynous in the bovines. The animal with a count greater than 40 ticks was treated with transcutaneous acaricide. The number of applications was 74% higher in 2019/20. In the year 2019/20, 6% and 3% of the animals received 5 and 6 applications, respectively. In the average of the years of evaluation, the animals received 1.8 doses of acaricides and 13% of the animals did not receive acaricide. In relation to the conventional model of treatment, the animals submitted to the selective treatment had an economy of acaricides in the order of 76%, 79%, 70% and 72%, in the



years 2017/18, 2018/19, 2019/20 and 2020 /21, respectively. In this way, selective treatment proves to be an efficient management in reducing the number of doses of acaricides applied annually with the potential to reduce environmental contamination and selection pressure in the generation of resistant populations.

**Keywords:** parasite control, bovine tick, ectoparasite



## **RUPTURA DO TENDÃO PRÉ-PÚBICO EM VACA NO SEMIÁRIDO DO RIO GRANDE DO NORTE: RELATO DE CASO**

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O tendão pré-púbico é uma estrutura anatômica que promove a inserção comum para os músculos abdominais e a linha alba. O rompimento do tendão pré-púbico ou desmorrexia pré-pubiana da prenhez é uma condição que pode afetar comumente éguas, entretanto rara em vacas e ovelhas, já que nessas o tendão pré-púbico recebe apoio adicional do tendão sub-pubiano. O objetivo desse trabalho é relatar um caso de ruptura do tendão pré-púbico em uma vaca, no semiárido do Rio Grande do Norte. Foi encaminhada ao abatedouro, uma vaca, 4 anos de idade, SRD, de aptidão leiteira, apresentando dilatação abdominal esquerda, alterações no membro esquerdo e dificuldade de locomoção. Na anamnese, o proprietário relatou o episódio de tentativa de monta natural com um reprodutor pesado, ocasionando a queda da vaca na segunda tentativa que, ao levantar-se, apresentou dificuldade de locomoção e em 24 horas o aumento de volume no lado esquerdo. Na propriedade, foram administradas uma dose de Oxitetraciclina e a cada 24 horas uma dose de Dexametasona. Passado o



período de carência, o animal foi encaminhado para o abate. Na inspeção pré-abate o animal apresentava-se em estação, com dificuldade para deitar-se e dor à locomoção, com parâmetros clínicos normais para a espécie. À palpação, perda da sustentação abdominal no lado esquerdo. Sendo submetida ao abate de emergência. Ao exame interno da carcaça, o animal não apresentava ascite. No sistema genital, o útero apresentava conformação normal, com vesícula gestacional de aproximadamente 1 mês. O rompimento do tendão pré-púbico ocorre geralmente no terço final da gestação devido gestações gemelares ou prolongadas, gigantismo fetal e hidropsia das membranas fetais, favorecendo o surgimento de edemaciação tensa e dolorosa, culminando na ruptura. Em vacas, a condição é rara devido ao reforço extra de sustentação, porém, quando ocorre, está relacionada às fêmeas com idade avançada e multíparas. Portanto, este caso veio trazer a informação de que o manejo reprodutivo adequado é de fundamental importância para evitar o surgimento dessa patologia em bovinos, principalmente nos casos de monta natural em que haja discrepâncias de tamanho e peso entre os reprodutores e as matrizes.

**Palavras-chave:** Patologia; Reprodução; Bovinos





## **RUPTURE OF PRE-PUBIC TENDON IN A COW IN THE SEMIARID REGION OF RIO GRANDE DO NORTE: CASE REPORT**

The prepubic tendon is an anatomical structure that provides a common insertion for the abdominal muscles and the linea alba. The rupture of the prepubic tendon, or prepubertal demorhexis of pregnancy, is a condition that can commonly affect mares, but is rare in cows and ewes, since in the latter the prepubic tendon receives additional support from the sub-pubic tendon. The objective of this study is to report a case of rupture of the prepubic tendon in a cow in the semi-arid region of Rio Grande do Norte. A 4-year-old non-breed dairy cow was referred to the slaughterhouse, presenting left abdominal dilatation, changes in the left limb and difficulty in locomotion. During anamnesis, the owner reported an episode of attempted natural mating with a heavy bull, causing the cow to fall on the second attempt. On the ranch, a dose of Oxytetracycline and every 24 hours a dose of Dexamethasone were administered. After the waiting period, the animal was sent to slaughterhouse. In the pre-slaughter inspection, the animal was in station, with difficulty lying down and pain on locomotion, with normal clinical parameters for the bovine species. On palpation, loss of abdominal support on the left side. The animal was submitted to emergency slaughter. On internal examination of the carcass, the animal did not present ascites. In the genital system, the uterus presented normal conformation, with gestational vesicle of approximately 1 month. The rupture of the pre-pubic tendon usually occurs in the final third of



gestation due to twin or prolonged pregnancies, fetal gigantism and hydrops of the fetal membranes, favoring the appearance of tense and painful edema, culminating in rupture. In cows, the condition is rare due to the extra support reinforcement, however, when it occurs, it is related to females with advanced age and multiparous. Therefore, this case brought the information that the adequate reproductive management is of fundamental importance to avoid the appearance of this pathology in bovines, mainly in cases of natural mounting in which there are size and weight discrepancies between sires and dams.

**Keywords:** Pathology; Reproduction; Cattle



## **SÍNDROME DO CORPO ESTRANHO METÁLICO EM VACA GIROLANDO: RELATO DE CASO**

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A reticulite traumática ou síndrome do corpo estranho metálico é uma injúria do trato digestivo, causada pela ingestão de corpos estranhos metálicos e pontiagudos gerando um complexo de enfermidades nos bovinos. Objetiva-se relatar um caso de síndrome do corpo estranho metálico em vaca Girolando, atendida no Hospital Veterinário da Universidade Federal do Espírito Santo. Há seis meses o animal apresentava queda da produção leiteira, redução do apetite, emagrecimento progressivo, queda no escore de condição corporal e relutância ao andar. Clinicamente estava apática com escore de condição corporal regular, mucosas externas pálidas, desidratação moderada, apetite caprichoso, tosse presente e discreta resposta a provas de dor, os demais parâmetros se apresentavam fisiológicos. Os achados ultrassonográficos do rim direito demonstraram ecogenicidade cortical aumentada e ecotextura discretamente heterogênea compatíveis com nefropatia. O hemograma constatou anemia normocítica normocrômica e hiperfibrinogenemia. Foram realizados três exames bioquímicos no intervalo de sete dias, que evidenciaram valores crescentes de



albumina, gama glutamil transferase, creatinina e ureia, somado a hiperglobulinemia e hiperproteinemia. Optou-se por realizar laparorrumenotomia exploratória esquerda que revelou aderências de rúmen, baço e parede do peritônio ao diafragma, assim como rim esquerdo aumentado de tamanho, devido aos achados transoperatórios não passíveis de correção, e o declínio do quadro clínico, o animal foi eutanasiado. A necropsia evidenciou peritonite fibrinohemorrágica difusa moderada, corpo estranho metálico de 2 cm (prego) fixado nas pregas reticulares, esplenomegalia, periesplenite fibrinosa, colicistite hemorrágica difusa severa, úlceras abomasais multifocais intensas, hepatomegalia moderada, nefrite crônica e hidronefrose. A microscopia relatou colecistite necrotizante multifocal intensa, congestão esplênica difusa severa, fígado com áreas multifocais em intensa quantidade de material amorfo, eosinofílico e acelular, rins com glomerulonefrite membranosa, glomeruloesclerose e fibroplastia intersticial multifocal moderada e infiltrado inflamatório intersticial multifocal discreto. Os danos renais crônicos e severos evidenciados pelo aumento dos biomarcadores da função renal, achados anatomopatológicos e exames de imagem, podem ser explicados devido a deposição de imunocomplexo causada pelo processo inflamatório intenso. Portanto, conclui-se a partir dos achados clínico patológicos, cirúrgicos e de imagem que o animal apresentava síndrome do corpo estranho metálico.

**Palavras-chave:** Corpo estranho metálico, reticulo peritonite, proteína de fase aguda, doenças digestivas



## **METALLIC FOREIGN BODY SYNDROME IN GIROLANDO COW: CASE REPORT**

Traumatic reticulitis or metallic foreign body syndrome is an injury to the digestive tract, caused by the ingestion of sharp and metallic foreign bodies, generating a complex of diseases in cattle. The objective is to report a case of metallic foreign body syndrome in a Girolando cow, treated at the Veterinary Hospital of the Federal University of Espírito Santo. Six months ago, the animal had a drop in milk production, reduced appetite, progressive weight loss, a drop in body condition score and reluctance to walk. Clinically, she was apathetic with a regular body condition score, pale external mucous membranes, moderate dehydration, capricious appetite, present cough and discreet response to pain tests, the other parameters were physiological. Ultrasound findings of the right kidney demonstrated increased cortical echogenicity and slightly heterogeneous echotexture consistent with nephropathy. The blood count showed normocytic normochromic anemia and hyperfibrinogenemia. Three biochemical tests were performed within seven days, which showed increasing values of albumin, gamma glutamyl transferase, creatinine and urea, in addition to hyperglobulinemia and hyperproteinemia. It was decided to perform a left exploratory laparotomy which revealed adhesions of the rumen, spleen and wall of the peritoneum to the diaphragm, as well as an enlarged left kidney, due to intraoperative findings that could not be corrected, and the decline of the clinical picture, the animal was euthanized. The necropsy showed moderate



diffuse fibrinohemorrhagic peritonitis, a 2 cm metallic foreign body (nail) fixed in the reticular folds, splenomegaly, fibrinous perisplenitis, severe diffuse hemorrhagic cholecystitis, intense multifocal abomasal ulcers, moderate hepatomegaly, chronic nephritis and hydronephrosis. Microscopy reported intense multifocal necrotizing cholecystitis, severe diffuse splenic congestion, liver with multifocal areas in intense amount of amorphous, eosinophilic and acellular material, kidneys with membranous glomerulonephritis, glomerulosclerosis and moderate multifocal interstitial fibroplasties and discrete multifocal interstitial inflammatory infiltrate. The chronic and severe kidney damage evidenced by the increase in renal function biomarkers, anatomopathological findings and imaging tests, can be explained due to the deposition of the immune complex caused by the intense inflammatory process. Therefore, it is concluded from the clinical, pathological, surgical and imaging findings that the animal had metallic foreign body syndrome.

**Keywords:** Metallic foreign body, peritonitis reticulum, acute phase protein, digestive diseases



## SUSCETIBILIDADE AOS ANTIMICROBIANOS E MONITORAMENTO DOS AGENTES ASSOCIADOS À MASTITE SUBCLÍNICA EM PROPRIEDADE RURAL DE GOIÂNIA, GOIÁS

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A qualidade do leite produzido é baseada em diversos fatores como a baixa contagem de células somáticas (CCS) e a mastite bovina representa um dos principais problemas sanitários na pecuária de leite. *Staphylococcus aureus*, *Streptococcus agalactiae* e *Mycoplasma bovis* destacam-se entre os agentes associados à mastite subclínica e a antibioticoterapia no período seco da vaca é o principal método de controle dessa enfermidade. Assim, o presente estudo teve como objetivo identificar os principais agentes associados à mastite subclínica e a sua resistência antimicrobiana em uma propriedade de gado leiteiro localizada em Goiânia, Goiás. Para a colheita do leite, foram selecionados os animais que apresentaram CCS acima de 200.000 células por mL de leite e realizou-se o *California Mastitis Test* (CMT) para a identificação dos quartos afetados. As amostras de leite (100µl) dos animais com mastite subclínica foram semeadas em ágar sangue ovino (5%) e ágar MacConkey e incubadas a 37°C, em condições de aerobiose, com leitura e identificação das colônias em 24, 48 e 72 horas. Os microrganismos isolados foram avaliados pela coloração de Gram e,



posteriormente, submetidos às provas bioquímicas. Após a identificação, realizou-se o teste de disco-difusão em ágar para avaliar a susceptibilidade antimicrobiana *in vitro*. Em um período de 3 meses, foram coletadas 12 amostras de um total de 48 de vacas em lactação do rebanho. Do total de 12 amostras coletadas, 33% dos isolados foram identificados como *Escherichia coli*, 25% como *Streptococcus sp*, 25% como *Staphylococcus aureus* e 16,5% como *Enterobacter aerogenes*. Foram testados 8 antimicrobianos para cada agente isolado e todos os microrganismos identificados apresentaram resistência a pelo menos dois antimicrobianos avaliados, resultando em resistência de no mínimo 25%. Os resultados do presente estudo evidenciam a importância das boas práticas de higiene na ordenha e sanidade da bovinocultura leiteira e reforçam a necessidade do uso consciente de antimicrobianos.

**Palavras- chave:** Mastite; sanidade; resistência; antimicrobianos





## SUSCEPTIBILITY TO ANTIMICROBIALS AND MONITORING OF AGENTS ASSOCIATED WITH SUBCLINICAL MASTITIS AT A FARM IN GOIÂNIA, GOIÁS

The quality of the produced milk is based on several factors such as low somatic cell count (SCC), and the bovine mast cell represents one of the health problems in dairy farming. *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma bovis* stand out among the agents associated with subclinical mastitis and antibiotic therapy in the dry period of the vacancy and it's the main method of controlling this disease. Thus, the present study aimed to identify the main agents associated with subclinical mastitis and their antimicrobial resistance in a dairy farm located in Goiânia, Goiás. For milk collection, animals that presented CCS above 200.000 cells per mL of milk were selected and the California Mastitis Test (CMT) was performed for identification of the affected quarter. Milk samples (100µl) from animals with subclinical mastitis were seeded on sheep blood agar (5%) and MacConkey agar and incubated at 37°C, under aerobic conditions, with reading and identification of colonies at 24, 48 and 72 hours. The isolated microorganisms were evaluated by Gram analysis and, later, selected for biochemical tests. After identification, the disk-diffusion test on agar was performed to evaluate the in vitro antimicrobial susceptibility. In a period of 3 months, 12 samples were collected from a total of 48 lactating cows from the herd. Of the 12 samples collected, 33% of the isolates were identified as *Escherichia coli*, 25% as *Streptococcus sp*, 25% as *Staphylococcus aureus* and



16.5% as *Enterobacter aerogenes*. At least 8 antimicrobials were tested for each isolated agent and all microorganisms showed resistance to at least two antimicrobials, resulting in resistance of 25%. The results of the study show the importance of good hygiene practices in milking and health of dairy cattle and reinforce the need for the conscious use of antimicrobials.

**Keywords:** mastitis; sanity; resistance; antimicrobials



## TAXA DE MORTALIDADE EM BEZERROS DA RAÇA GIROLANDO CRIADAS NO TRÓPICO ÚMIDO AMAZÔNICO

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Elevadas taxas de mortalidade de bezerros são as principais causas de perdas econômicas na produção leiteira e doenças como diarreia, onfalopatias e síndrome respiratória, contribuem, significativamente para o aumento dessas taxas. Com este trabalho, objetivou-se descrever a taxa de mortalidade em bezerros girolandos criados no trópico úmido amazônico. Em uma propriedade parceira localizada no município de Paragominas-PA, no período entre dezembro de 2021 a julho de 2022, 80 bezerros, da raça girolando (28 machos e 52 fêmeas) foram aleatoriamente escolhidos, em delineamento casualizado e acompanhados desde o nascimento até os 60 dias de idade. A propriedade possuía banco de colostro e ao nascimento de um bezerro era fornecido colostro descongelado em banho-maria a temperatura média de 35 a 40 °C, com grau brix de 25%. Quando necessário o colostro era enriquecido com colostro artificial em pó até atingir o grau brix igual a 25%. Para prevenção de onfalopatias o



cordão umbilical era tratado com iodo 10% até a mumificação e queda do coto umbilical externo. A taxa de mortalidade foi obtida pela divisão entre o número de bezerros mortos, pelo de bezerros nascidos vivos, multiplicado por 100. Pelos resultados obtidos, foi possível observar episódios de diarreia, onfalopatias e doenças respiratórias, que culminaram em uma taxa de mortalidade de bezerros de 6%. Esses valores encontram-se acima dos valores de referência tidos como *Padrão Ouro de criação de bezerras e novilhas leiteiras* (Alta Genetics) que cita uma porcentagem menor que 3% para a mortalidade de bezerras, na fase de aleitamento. Apesar dos bezerros serem adequadamente colostrados e monitorados quanto à prevenção de doenças do umbigo, a alta taxa de mortalidade dos bezerros pode estar ligada aos fatores que favorecem a morbidade dessas doenças, além das respiratórias, que acometem os bezerros durante a fase de aleitamento. Assim, falhas em outras medidas que porventura concorram para o surgimento dessas doenças devem ser investigadas.

**Palavras-chave:** Amazônia, Bezerro, Bovinocultura leiteira, Neonato, Saúde



## MORTALITY RATE IN GIROLANDO CALVES BREEDED IN THE AMAZON MOIST TROPIC

High calf mortality rates are the main causes of economic losses in dairy production and diseases such as diarrhea, omphalopathies and respiratory syndrome, contribute significantly to the increase in these rates. The objective of this work was to describe the mortality rate in girolando calves raised in the Amazonian humid tropics. In a partner property located in the municipality of Paragominas-PA, from December 2021 to July 2022, 80 girolando calves (28 males and 52 females) were randomly chosen, in a randomized design and followed from birth to 60 days old. The property had a colostrum bank and at the birth of a calf, colostrum thawed in a water bath at an average temperature of 35 to 40 °C, with a Brix degree of 25%. When necessary, the colostrum was enriched with artificial colostrum powder until reaching a brix degree of 25%. To prevent omphalopathies, the umbilical cord was treated with 10% iodine until mummification and fall of the external umbilical stump. The mortality rate is obtained by dividing the number of dead calves by the number of live-born calves, multiplied by 100. From the results obtained, it was possible to observe episodes of diarrhea, omphalopathies and respiratory diseases, which culminated in a mortality rate of calves of 6%. These values are above the reference values taken as *the Gold Standard for the creation of dairy calves and heifers* (Alta Genetics) which cites a percentage lower than 3% for calf mortality in the suckling phase. Although calves are adequately colostrated and monitored for the prevention of navel diseases, the high mortality rate of calves may be linked to factors that favor



the morbidity of these diseases, in addition to respiratory diseases, which affect calves during the lactation phase. Thus, failures in other measures that may contribute to the emergence of these diseases should be investigated.

**Keywords:** Amazon, Calf, Dairy cattle, Health, Neonate



## **TÉCNICA DE APLICAÇÃO INTRABOMASAL DE SUCRALFATO PARA TRATAMENTO DE ÚLCERA DE ABOMASO – RELATO DE CASO**

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Foi atendido no Complexo Veterinário da Universidade um bovino, fêmea, 30 anos de idade, sem raça definida, pesando 548 kg. Cinco dias após ser submetida à ruminotomia, animal começou a apresentar diarreia com melena, bruxismo intenso, escoiceamento de abdômen, indicando presença de úlcera de abomaso. Inicialmente foi instituído tratamento com cimetidina intravenosa (16 mg/kg), por 10 dias, sem sinais de melhora. Como segunda alternativa foi instituída a aplicação transmural de sucralfato no abomaso. A técnica para a aplicação envolveu o acesso percutâneo ao abomaso para a introdução de uma sonda de Foley 16. O procedimento foi realizado com o animal em estação sob anestesia local plano-a-plano. Em cada camada tecidual foram aplicados entre 3-5 mL de anestésico local (Lidocaína<sup>®</sup> 2%). O acesso foi realizado no abdômen na face ventro-lateral direita, caudal à última costela, aproximadamente a 10 cm



da linha mediana ventral. Foi realizado uma incisão cutânea de 5 cm, seguido da divulsão romba dos tecidos moles até a localização dos músculos oblíquo abdominal externo e interno. Por esses músculos, a divulsão avançou com pinça hemostática Kelly curva até adentrar o abdômen. O processo foi seguido com uma pequena perfuração do peritônio para a completa visualização da parede lateral do abomaso. Seguidamente, o abomaso foi lancetado com uma lâmina de bisturi nº 11 para a introdução da sonda, que foi direcionada para dentro do abomaso com uma pinça Kelly curva, mantida posteriormente aberta para a introdução e avanço da sonda até uma profundidade de 10 cm. O balão da sonda foi inflado para que isolasse a área e impedisse seu deslocamento, posteriormente essa foi fixada a pele através de fio de nylon 0-0, e trançado sobre a sonda como formato de “fita de Bailarina”. Pela sonda, foi introduzido 40 mL de sucralfato (2g/frasco) BID por 5 dias. Ao longo dos cinco dias as manifestações clínicas de úlcera apresentaram remissão. Quinze dias após, animal foi eutanasiado por outros motivos, na necrópsia pode ser observada a cicatrização das úlceras, demonstrando que o procedimento obteve sucesso para a cura das mesmas.

**Palavras-chave:** Bovinocultura, cirurgia, sanidade, úlcera abomasal





## **SUCRALFATE INTRABOMASAL APPLICATION TECHNIQUE FOR ABOMASAL ULCER TREATMENT - CASE REPORT**

A 30-year-old female bovine animal of undefined breed, weighing 548 kg, was treated at the Veterinary Complex of the University. Five days after undergoing ruminotomy, the animal began to present diarrhea with melena, intense bruxism, scaling of the abdomen, indicating the presence of abomasal ulcer. Initially, treatment was instituted with intravenous cimetidine (16 mg/kg) for 10 days, with no signs of improvement. As a second alternative, transmural application of sucralfate to the abomasum was instituted. The technique for application involved percutaneous access to the abomasum for the introduction of a Foley 16 probe. The procedure was performed with the animal in station under local plane-to-plane anesthesia. Between 3-5 mL of local anesthetic (Lidocaine® 2%) was applied to each tissue layer. Access was performed in the abdomen on the right ventro-lateral side, caudal to the last rib, approximately 10 cm from the ventral midline. A 5-cm skin incision was made, followed by blunt divulsion of the soft tissues up to the location of the external and internal oblique abdominal muscles. Through these muscles, the divulsion was advanced with curved Kelly hemostat until it entered the abdomen. The process was followed with a small perforation of the peritoneum for complete visualization of the lateral wall of the abomasum. The abomasum was then lanced with a #11 scalpel blade for introduction of the probe, which was directed into the abomasum with curved Kelly forceps and then held open for introduction and advancement of the probe to a depth of 10 cm. The balloon of the probe was inflated to isolate the area and prevent its



displacement, and then it was attached to the skin using 0-0 nylon thread and braided over the probe in the form of a "ballerina's ribbon". Through the probe, 40 mL of sucralfate (2g/bottle) BID was introduced for 5 days. Over the five days the clinical manifestations of ulceration showed remission. Fifteen days later, the animal was euthanized for other reasons, and in the necropsy the healing of the ulcers could be observed, demonstrating that the procedure was successful in healing them.

**Keywords:** Cattle, surgery, sanity, abomasal ulcer



## **TRANSFERÊNCIA DE PLASMA SANGUÍNEO EM BEZERRO COM FALHA NA TRANSFERÊNCIA DE IMUNIDADE PASSIVA - RELATO DE CASO**

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A placenta sinepiteliocorial das vacas não possibilita ideal transferência materno-fetal de imunoglobulinas durante a gestação, sendo essa transferência realizada através do colostro. Quando não há ingestão em quantidade, qualidade e tempo adequados, ocorre a falha de transferência de imunidade passiva (FTIP), sendo esta uma das principais causas de mortalidade de bezerros neonatos. O objetivo deste trabalho é relatar o caso de FTIP em um bovino macho, 2 dias de idade, da raça Braford. O bezerro foi encontrado no campo em decúbito lateral e foi levado para atendimento. Na anamnese foi informado que a mãe não permitiu que o bezerro chegasse próximo dela e que a mesma possuía o úbere pouco desenvolvido. Ao exame físico, observou-se fraqueza, dificuldade de ficar em estação, flanco fundo, FC = 110bpm, taquipneia (FR = 60mrpm), febre (T°C = 40°C), desidratação leve (5%), e presença de miíase no umbigo. A suspeita foi de FTIP. Foi realizada coleta de sangue para avaliação da proteína sérica total



(PST) obtendo o resultado de 3,4g/dL, confirmando suspeita de FTIP. Como tratamento, foi instituído 2L de drench para bezerros via oral (VO), 10mL de suplemento aminoácido VO durante três dias, limpeza do umbigo com clorexidine a 0,5% e aplicação tópica de iodo a 2% por 4 dias. No primeiro dia de internação, além da hidratação realizou-se transfusão de 280mL de plasma bovino obtidos a partir de 548mL de sangue de uma doadora. Posteriormente, o paciente começou a apresentar melhora clínica, ficou em estação sem auxílio e ingeriu 2L de leite com vigor. Dois dias após a transferência de plasma realizou-se a determinação da PST, obtendo-se valor de 5,2g/dL. Para considerar a transferência de imunidade passiva como boa, o valor mínimo de referência de proteína sérica deve ser de 5,8g/dL. Ainda que o resultado de PST obtido com a transferência de plasma tenha sido abaixo do desejado, deve-se considerar que o paciente permaneceu internado e não apresentou nenhuma enfermidade até os 5 meses de idade. Conclui-se que a transferência de plasma é uma alternativa eficiente para aumentar a concentração sérica de imunoglobulinas e promover imunidade ao bezerro neonato.

**Palavras-chave:** Imunidade, anticorpos, neonato



## **BLOOD PLASMA TRANSFERENCE IN CALF WITH FAILURE OF PASSIVE IMMUNITY TRANSFER - CASE REPORT**

As the cow's epitheliochorial placenta does not grant optimal maternal-fetal transfer of immunoglobulins during pregnancy, it must occur through the colostrum. If there is no adequate colostrum intake in either amount, quality, or time, there will be failure of passive immunity transfer (FPT), one of the main causes of newborn calves' mortality. The work aimed to report the FPT case in a male Hereford x Nelore crossbred calf, two days old. The calf was identified on the field in lateral decubitus and was taken in for medical assistance. During the anamnesis it was informed that the mother did not allow the calf's approach and that its udder was underdeveloped. At the physical exam it was noted weakness, trouble standing, deep flank, HR = 110bpm, tachypnea ( $f = 60$ impm), mild fever ( $T^{\circ}\text{C} = 40^{\circ}\text{C}$ ), slight dehydration (5%), and had myiasis on the navel. The suspected diagnosis was FPT. A blood sample was collected to evaluate total serum protein (TSP) resulting in 3,4g/dL, confirming the FPT (TSP <5,1g/dL until 48h of birth). The treatment established was 2L of drench for calves orally, 10mL of aminoacid supplement orally for three days, navel disinfection with chlorhexidine 0,5% and topic iodine 2% for 4 days. On the first day of hospitalization, besides intravenous fluids for hydration, it was performed a transfusion of 280mL of bovine blood plasma obtained from 548mL of whole blood from a donor. Afterwards the patient began to present clinical improvement, stood still without assistance and ingested 2L of milk vigorously. Two days after blood plasma transference the TSP was evaluated getting 5,2g/dL as the result.



A good transfer of passive immunity has 5,8g/dL as the minimum reference value of serum protein. Even though the result reached is lower than intended it must be considered that the patient was kept hospitalized and did not present any diseases until five months old. In conclusion, blood plasma transfer is an efficient alternative to raise the serum concentration of immunoglobulins and to promote immunity to the newborn calf.

**Keywords:** Immunity, antibodies, newborn



## TRANSMISSÃO TRANSPLACENTÁRIA DE BABESIOSE BOVINA: RELATO DE CASO

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Dentre os métodos diagnósticos para babesiose bovina descritos na literatura incluem-se a descrição clínica, alterações necroscópicas, *imprint* com colorações diferenciais e PCR. Objetivou-se, neste estudo, relatar a transmissão transplacentária de *Babesia bovis* em um feto bovino abortado congelado e *Babesia bigemina* em um bezerro natimorto refrigerado. Observou-se na necropsia: cérebro hiperêmico, cor de cereja; mucosas, gordura corpórea e visceral ictéricas; linfadenomegalia pré-escapular; reação de polpa branca esplênica; petéquias e sufusões no epicárdio; hepatomegalia. Os materiais coletados para estudo foram: 1) *imprints* de cérebro, fígado e baço fixados em álcool 70%, com posterior coloração por kit panótico rápido modificado; 2) fragmentos desses órgãos fixados em formol 10% foram processados por histotécnica convencional (desidratação, diafanização, embebição e emblocagem em parafina), e cortes desparafinados de um micrômetro foram corados por hematoxilina/eosina, e por coloração diferencial do panótico modificado com eosina histológica; 3) cérebro, baço, fígado, rim e coração para



ensaio por biologia molecular via PCR. Tanto o *imprint* cerebral quanto o histopatológico nas lâminas desparafinadas do cérebro do neonato refrigerado permitiram a visualização de *Babesia* spp. no citoplasma das hemácias localizadas dentro de capilares sanguíneos. Entretanto, o feto abortado apresentava-se congelado e sangue sofreu hemólise, e por esse motivo a análise das hemácias no *imprint* cerebral e no histopatológico foi inviável. Foi imprescindível o diagnóstico confirmatório por PCR com alvo nos genes rap-1 de *Babesia bovis* e *Babesia bigemina*, permitindo a detecção de *Babesia bovis* nos fragmentos de SNC, fígado, rim e coração do feto abortado e de *Babesia bigemina* no pool de órgãos do natimorto. A utilização de *imprint* e histopatologia em cortes desparafinados de cérebro possibilitaram observar os hematozoários em carcaça não congelada, porém ressalta-se que somente a PCR permitiu a identificação de *Babesia bovis* no feto abortado e de *Babesia bigemina* no natimorto, confirmando a transmissão placentária desses parasitas. Os resultados corroboram com a literatura, de forma a considerar a inclusão da babesiose no diagnóstico diferencial em doenças reprodutivas dos bovinos e ressaltar a importância da requisição de técnicas diversificadas complementares para melhor identificação das afecções que acometem os rebanhos, bem como suas atualizações científicas.

**Palavras-chave:** *Babesia bovis*; *Babesia bigemina*; Feto abortado; Natimorto; Métodos diagnósticos





## TRANSPLACENTAL TRANSMISSION OF BOVINE BABESIOSIS: CASE REPORT

Among the diagnostic methods for bovine babesiosis described in the literature are clinical description, necroscopic alterations, imprint with differential stain colour and PCR. The aim of this study was to report the transplacental transmission of *Babesia bovis* in frozen aborted bovine fetal and *Babesia bigemina* in a refrigerated stillbirth cattle. At necropsy was observed: hyperemic, cherry-colored brain; mucous membranes, body fat and visceral fat icterical; prescapular lymphadenomegaly; splenic white pulp reaction; petechiae and suffusions in the epicardium; hepatomegaly. Materials collected for study: 1) brain, liver and spleen imprints fixed in alcohol 70%, with subsequent staining by modified rapid panoptic kit; 2) fragments of these organs fixed in 10% formalin were processed by conventional histotechnique (dehydration, diaphanization, soaking, and paraffin embedding), and one micrometer dewaxed sections were stained by hematoxylin/eosin, and by differential staining of the modified panopticon with histological eosin; 3) brain, spleen, liver, kidney, and heart for molecular biology assay by PCR. Both brain imprinting and histopathology on dewaxed slides of the brain of the chilled neonate allowed visualization of *Babesia* spp. in the cytoplasm of RBCs located within blood capillaries. However, the aborted fetus was frozen and blood was hemolyzed, so the brain imprint and histopathological analysis of the RBCs were unviable. The confirmatory diagnosis by PCR targeting the rap-1 genes of *Babesia bovis* and *Babesia bigemina* was



indispensable, allowing the detection of *Babesia bovis* in the fragments of CNS, liver, kidney and heart of the aborted fetus, and of *Babesia bigemina* in the organ fragments of the calf stillborn. The use of imprint and histopathology in deparaffinized brain slides allowed the observation of hematozoa in non-frozen carcasses. However, is important to emphasize that only PCR allowed the identification of *Babesia bovis* in the aborted fetus and *Babesia bigemina* in the stillbirth, confirming the placental transmission of those parasites. The results corroborate with literature, in way to consider the inclusion of babesiosis in the differential diagnosis in reproductive diseases of bovines, and to emphasizing the importance about request diversified complementary techniques for a better identification of the disorders that affect the herd, as well as their scientific updates.

**Keywords:** *Babesia bovis*; *Babesia bigemina*; Fetus aborted; Stillborn; Diagnostic methods



## USO DE DIETA SUPLEMENTAR PROTÉICA NA PRODUÇÃO ANIMAL: PROCESSOS BIOLÓGICOS AFETADOS NO PROTEOMA SALIVAR E IMPACTOS NO PERIODONTO DE BOVINOS

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A saliva é um fluído rico em componentes que demonstra possíveis alterações locais e sistêmicas, como as gengivites e outras doenças periodontais. Em ruminantes, essas enfermidades afetam a produção e podem estar relacionadas à dieta. O objetivo do presente estudo foi o de caracterizar os principais processos biológicos afetados no proteoma salivar de bovinos e os possíveis efeitos no periodonto de animais confinados com suplemento proteico (SP) e feno (F) de *Panicum* produzido em área reformada. Ao grupo SP (n=6), foi ofertado feno e suplemento proteico. Ao grupo F (n=6), a dieta restringiu-se ao



fenô por um período de 60 dias. O status clínico bucal foi estabelecido por exame clínico intra-bucal semanal e sondagem dos incisivos e a saliva foi coletada aos 20 e 60 dias. A classificação funcional dos processos com termo mais significativo foi realizada de acordo com o Gene Ontology (aplicativo ClueGo do software Cytoscape) e o número de acesso às proteínas foi fornecido pelo banco de dados *Uniprot* para espécie *Bos taurus*. Todos animais desenvolveram gengivite, entretanto a média de sítios dentários afetados diferiu entre os grupos: no grupo HP tendeu à superioridade aos 60 dias ( $1,33 \pm 0,42$ ;  $p=0,404$ ); e no grupo F foi superior aos 20 dias ( $3,83 \pm 1,40$ ;  $p=0,003$ ). Aos 20 dias foram encontrados sete processos biológicos alterados na saliva dos bovinos, destacando-se entre as categorias com os maiores percentuais de genes resposta humoral antimicrobiana (19%) envolvendo as proteínas *lactoperoxidase*, *lactotransferrina*, *cadeia beta de fibrinogênio*, *proteína de reconhecimento de peptidoglicano 1* e *albumina*. Aos 60 dias, nove processos biológicos alterados foram identificados na saliva dos animais, destacando-se o processo de resposta humoral antimicrobiana (14%) (compreendendo as mesmas proteínas identificadas aos 20 dias e *catelicidina-5*); e resposta inflamatória aguda (11%) envolvendo *complemento C3*, *deoxyribonuclease*, *haptoglobina* e *alpha-1-ácido-glicoproteína*. Esses resultados preliminares permitem verificar a expressão de proteínas multifuncionais (particularmente envolvidas em respostas anti-inflamatórias e antimicrobianas), demonstrando que o perfil proteico salivar de bovinos se altera quando os animais são expostos a dietas proteicas de alto desempenho.

**Palavras-Chave:** bovinos, gengivite, proteoma salivar



## **USE OF SUPPLEMENTARY PROTEIN DIET IN ANIMAL PRODUCTION: BIOLOGICAL PROCESSES AFFECTED IN THE SALIVAR PROTEOMA AND IMPACTS IN THE PERIODONTAL OF CATTLE**

Saliva is a fluid rich in components that demonstrates possible local and systemic changes, such as gingivitis and other periodontal diseases. In ruminants, these diseases affect production and may be related to diet. The aim of the present study was to characterize the main biological processes affected in the salivary proteome of cattle and the possible effects on the periodontium of animals confined with protein supplement (PS) and Panicum hay (H) produced in a reformed area. The PS group (n=6) was offered hay and protein supplement. In group H (n=6), the diet was restricted to hay for a period of 60 days. Oral clinical status was established by weekly intraoral clinical examination and incisor probing and saliva was collected at 20 and 60 days. The functional classification of the processes with the most significant term was performed according to the Gene Ontology (ClueGo application of the Cytoscape software) and the accession number to the proteins was provided by the Uniprot database for the *Bos taurus* species. All animals developed gingivitis, however the mean number of affected dental sites differed between groups: in the PS group it tended to be superior at 60 days ( $1.33 \pm 0.42$ ;  $p=0.404$ ); and in group H it was higher at 20 days ( $3.83 \pm 1.40$ ;  $p=0.003$ ). At 20 days, seven altered biological processes were found in the saliva of cattle, standing out among the categories with the highest



percentages of humoral antimicrobial response genes (19%) involving the proteins lactoperoxidase, lactotransferrin, fibrinogen beta chain, peptidoglycan recognition protein 1 and albumin. At 60 days, nine altered biological processes were identified in the animals' saliva, highlighting the antimicrobial humoral response process (14%) (comprising the same proteins identified at 20 days and cathelicidin-5); and acute inflammatory response (11%) involving Complement C3, deoxyribonuclease, haptoglobin and alpha-1-acid-glycoprotein. These preliminary results allow us to verify the expression of multifunctional proteins (particularly involved in anti-inflammatory and antimicrobial responses), demonstrating that the salivary protein profile of cattle changes when animals are exposed to high-performance protein diets.

**Keywords:** cattle, gingivitis, salivary proteome



## **VALIDAÇÃO DE QUESTIONÁRIO PARA AVALIAÇÃO DO CONTROLE DE MASTITE E QUALIDADE DO LEITE BOVINO NO SUDOESTE PARANAENSE**

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A bovinocultura leiteira destaca-se no agronegócio brasileiro e o Estado do Paraná consolidou-se como segundo maior produtor de leite do país, com significativo crescimento na região sudoeste. A mastite é responsável pelas maiores perdas do setor. Desta forma, o estudo objetivou avaliar a confiabilidade de um questionário sobre controle e qualidade do leite no Sudoeste paranaense e validá-lo, para que seja aplicado na obtenção de dados que demonstrem as características produtivas da região. A pesquisa foi submetida e aprovada no Comitê de Ética em Pesquisa da Universidade Federal da Fronteira Sul – Campus Realeza (UFFS). O estudo foi dividido em duas etapas: 1. Validação do questionário; 2: Entrevista com produtores. Para a validação, o questionário foi



enviado a vinte produtores da região Sudoeste do Paraná e cinquenta discentes do curso de Medicina Veterinária da UFFS. Após essa etapa foram entrevistados 55 produtores, residentes nos municípios de Realeza, Salto do Lontra, São João, Coronel Vivida, Chopinzinho, Pato Branco, Sulina e Honório Serpa. O questionário foi composto por 37 questões abrangendo os seguintes tópicos: Características do rebanho; Práticas de biossegurança; Saúde do úbere; Controle de mastite e procedimentos de ordenha; Manejo das vacas secas. O mesmo foi aplicado em dois momentos, com intervalo de um mês, a fim de avaliar a confiabilidade das respostas. Para análise dos resultados foi aplicado o teste de correlação de *Pearson*, entre o momento um e dois, sendo considerada perfeita correlação ( $r = 1$ ). Os dados foram analisados pelo programa SAS® 2001, e apresentados por uma Tabela Dinâmica (*Pivot Table*), para comparação entre as variáveis presentes no questionário (produção média do rebanho, contagem de células somáticas, contagem bacteriana total e *California Mastitis Test*). Das 37 questões 72,97% apresentaram perfeita correlação ( $r = 1$ ), 18,91% os valores de  $r$  entre 0,95 e 0,99 e 8,1% os valores de  $r$  entre 0,90 e 0,94, sendo consideradas fortes correlações. A partir desses resultados confirma-se a confiabilidade e reprodutibilidade do questionário, tornando-o apto a ser aplicado de forma segura em estudos posteriores, podendo ser utilizado com um maior número de produtores e em diferentes locais.

**Palavras-chave:** Bovinocultura leiteira. Confiabilidade. Produtores leiteiros. Reprodutibilidade





## **VALIDATION OF A QUESTIONNAIRE TO EVALUATE MASTITIS CONTROL AND BOVINE MILK QUALITY IN THE SOUTHWEST OF PARANÁ**

Dairy cattle stands out in Brazilian agribusiness and the State of Paraná has consolidated itself as the second largest milk producer in the country, with significant growth in the southwest region. Mastitis is responsible for the biggest losses in the sector. Thus, the study aimed evaluate the reliability of a questionnaire on control and quality of milk in the Southwest of Paraná and validate it, so that it can be applied to obtain data that demonstrate the productive characteristics of the region. The research was submitted and approved by the Research Ethics Committee of the Federal University of Fronteira Sul – Campus Realeza (UFFS). The study was divided into two stages: 1. Validation of the questionnaire; 2: Interview with producers. For validation, the questionnaire was sent to twenty producers in the Southwest region of Paraná and fifty students of the Veterinary Medicine course at UFFS. After this stage, 55 producers were interviewed, living in the municipalities of Realeza, Salto do Lontra, São João, Coronel Vivida, Chopinzinho, Pato Branco, Sulina and Honório Serpa. The questionnaire consisted of 37 questions covering the following topics: Herd characteristics; Biosecurity practices; Udder health; Mastitis control and milking procedures; Dry cow management. The same was applied in two moments, with an interval of one month, in order to evaluate the reliability of the answers. To



analyze the results, Pearson's correlation test was applied, between moments one and two, being considered perfect correlation ( $r = 1$ ). The data were analyzed using the SAS® 2001 program, and presented by a Dynamic Table (Pivot Table), for comparison between the variables present in the questionnaire (average herd production, somatic cell count, total bacterial count and California Mastitis Test). Of the 37 questions, 72.97% showed perfect correlation ( $r = 1$ ), 18.91%  $r$  values between 0.95 and 0.99 and 8.1%  $r$  values between 0.90 and 0.94, being considered strong correlations. Based on these results, the reliability and reproducibility of the questionnaire are confirmed, making it able to be safely applied in further studies, allowing it to be used with a greater number of producers and in different locations.

**Keywords:** Dairy cattle. Reliability. Dairy producers. Reproducibility



## VARIAÇÃO DA PREVALÊNCIA DA MASTITE SUBCLÍNICA POR *Staphylococcus aureus* E *Streptococcus agalactiae* DURANTE BLITZ TERAPIA E SEUS IMPACTOS NO DIAGNÓSTICO

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*Staphylococcus aureus* e *Streptococcus agalactiae* são importantes patógenos da mastite contagiosa. Além de possuírem a mesma forma de infecção, estas bactérias causam mastite subclínica crônica, porém o tratamento para cada é diferente. *S. agalactiae* pode ser tratado com Blitz terapia, um protocolo de administração de antibióticos, enquanto o controle sugerido para *S. aureus* é o descarte de vacas cronicamente infectadas. Este estudo teve como objetivo a avaliação da variação da prevalência de *S. aureus* e *S. agalactiae* durante a implementação da Blitz terapia em um rebanho leiteiro. Os procedimentos foram aprovados pelo Comitê de Ética e Uso Animal da Universidade Federal Fluminense (protocolo 9683100719). Um rebanho foi selecionado devido a presença de *S. aureus* e *S. agalactiae* in leite de tanque de expansão e alta



contagem de células somáticas (CCS). O rebanho é localizado na região da Zona da Mata, no estado de Minas Gerais, Brasil. Após a seleção, amostras compostas de leite foram coletadas de todas as vacas em lactação (159 animais). A cultura do leite foi processada de acordo com o protocolo do National Mastitis Council. Todos os animais que obtiveram resultados positivos para *S. agalactiae* foram submetidos a Blitz terapia com cloxacilina e ampicilina em todos os tetos, durante 3 vezes (cada 12 horas). Após 21 dias, um novo procedimento de coleta foi realizado em todas as vacas lactantes. Este protocolo de coleta, cultura do leite e blitz terapia foi executado 5 vezes, durante 5 meses. Condutas apropriadas para a ordenha foram implementadas para prevenir novas infecções intramamárias. A prevalência de *S. aureus* e *S. agalactiae* foi estimada durante a blitz terapia. Durante o estudo, a prevalência de *S. agalactiae* variou de 61,6% para 3,8%, diminuindo em 57,8%. Enquanto isso, a prevalência de *S. aureus* variou de 28,3% para 35,0%, aumentando em 6,2%. Embora a blitz terapia seja eficaz para a eliminar *S. agalactiae*, curiosamente a prevalência de *S. aureus* aumentou mesmo com a implementação de medidas de controle. Isto sugere que a alta prevalência de *S. agalactiae* no início do estudo pode ter mascarado o diagnóstico de *S. aureus*.

**Palavras-chave:** Diagnóstico, Infecção intramamária, Epidemiologia, Antibioticoterapia



## PREVALENCE VARIATION OF SUBCLINICAL MASTITIS BY *Staphylococcus aureus* E *Streptococcus agalactiae* DURING BLITZ THERAPY AND ITS IMPACTS ON DIAGNOSIS

*Staphylococcus aureus* and *Streptococcus agalactiae* are important mastitis contagious pathogens. Besides those bacteria have the same form of infection, it causes chronic subclinical mastitis and their treatment is different. *S. agalactiae* can be treated with blitz therapy, a protocol of antibiotic administration, while the suggested control to *S. aureus* is culling the chronic infected cows. This study aimed to evaluate variation of *S. aureus* and *S. agalactiae* prevalence during blitz therapy implementation in a Holstein dairy herd. Procedures were approved by the Ethics and Animal Use Committee of Federal Fluminense University (protocol 9683100719). One herd was selected due to the presence of *S. aureus* and *S. agalactiae* in bulk milk tank and high somatic cell count (SCC). The herd was located in the Zona da Mata region in Minas Gerais state, Brazil. After selection, composed milk samples from all lactating cows (159 animals) were collected. Milk culture was performed according by National Mastitis Council protocol. All animals that had positive results to *S. agalactiae* were submitted to blitz therapy with cloxacilin and ampicilin. in all teats for three times (each 12 hours). After 21 days, a new collection procedure was performed in all lactating cows. This protocol of sampling collection, milk culture and treatment of positive cows to *S. agalactiae* was performed 5 times, during 5 months. Proper conducts during milking were proposed to prevent new intramammary infections. The prevalence



of *S. aureus* and *S. agalactiae* were estimated during blitz therapy. During the study, *S. agalactiae* prevalence ranged from 61.6% to 3.8%, decreasing in 58.7%. Meanwhile, *S. aureus* prevalence varied from 28.3% to 35.0%, rising 6.2%. Although blitz therapy was effective to eliminate *S. agalactiae* intramammary infections, curiously, *S. aureus* prevalence raised even with the implementation of control measures. It can suggest that the high prevalence of *S. agalactiae* in the beginning of the study could be impaired *S. aureus* diagnosis.

**Keywords:** Diagnosis, Intramammary infection, Epidemiology, Antibiotic Treatment