NATHÁLIA DECARIS

Bayesian Framework of the sensitivity and specificity of five pneumonia detection methods in pre weaning calves

São Paulo

2021

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Dissertation submitted to the Postgraduate Program in Veterinary Clinic of the School of Veterinary Medicine and Animal Science of University of São Paulo to obtain the Master's degree in Sciences.

Departament:
Clinical Medicine

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Advisor:

Prof. Viviani Gomes Ph.D.

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Comissão de Ética no Uso de Animais
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CERTIFICADO

Certificamos que a proposta intitulada "Análise Bayesiana da sensibilidade e especificidade de cinco métodos de detecção de broncopneumonia em bezerras na fase de aleitamento", protocolada sob o CEUA nº 5689310119 (ID 006864), sob a responsabilidade de **Viviani Gomes** *e equipe; Nathalia Decaris* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 14/08/2019.

We certify that the proposal "Bayesian Framework of the sensitivity and specificity of five pneumonia detection methods in pre weaning calves", utilizing 100 Bovines (100 females), protocol number CEUA 5689310119 (ID 006864), under the responsibility of **Viviani Gomes** *and team; Nathalia Decaris* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 08/14/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 06/2019 a 08/2019 Área: Clínica Médica Veterinária

Origem:	Animais de proprietários						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	30 a 80 dias	N:	100
Linhagem:	Holandesa			Peso:	50 a 150 kg		

Local do experimento: A parte experimental a campo será realizada na Fazenda Colorado, situada em Araras (SP). As análises laboratoriais serão realizadas no laboratório do departamento de Clínica Médica Veterinária (VCM), da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (USP)

São Paulo, 07 de junho de 2021

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes
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Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

EVALUATION FORM

Autor: DECARIS, Nathália

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Date: _____/_____/_____

Committee Members

Prof. ____

Institution: ______________________________Decision: ______________________

Prof. ____

Institution: ______________________________Decision: ______________________

Prof. ____

Institution: ______________________________Decision: ______________________

DEDICATION

To my parents, Marcelo and Milena for guiding me to not the easier way, but certainly the most grateful: the education.

To Pedro, my best choice and company every day.

To Professor Viviani Gomes and Gecria Team for all times of hard work that edifies.

In Memoriam Professor Fernando José Benesi an inspiration in the Buiatrics and an evolved soul that we had the privilege to be together.

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RESUMO

DECARIS, N. **Análise Bayesiana da sensibilidade e especificidade de cinco métodos de detecção de broncopneumonia em bezerras na fase de aleitamento.** 64 p. Dissertação (Mestrado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2021.

A broncopneumonia em bezerras é uma doença multifatorial, que gera prejuízos econômicos em curto e longo prazo, sendo a detecção precoce um fator crucial em relação a resposta terapêutica, gravidade da doença e mortalidade. A partir desta demanda, escores clínicos têm sido desenvolvidos na América do Norte para triagem dos animais a campo, além do incremento do uso da ultrassonografia torácica para a detecção de lesões de consolidação do parênquima pulmonar. Apesar das diferentes condições climáticas e do próprio sistema de criação, os métodos de diagnóstico padronizados na América do Norte têm sido aplicados em países subtropicais e tropicais. Diante do exposto, esta pesquisa avaliou a acurácia de 5 métodos de diagnóstico de broncopneumonias em bezerras da raça Holandesa submetidas a diferentes sistemas de criação em zona subtropical. Os testes utilizados para o diagnóstico das broncopneumonias foram ultrassonografia torácica (UST, positiva se a profundidade da consolidação ≥1cm), auscultação (Ausc, positivo na presença de crepitações, sibilos e ausência de sons), escore de Wisconsin (Wisc, positivo se ≥4), escore Californiano (Calif, positivo se ≥5) e concentração de haptoglobina sérica (Hap, positivo se ≥15 mg/dL). Bezerras de 17 fazendas comerciais do estado de São Paulo, com 30 dias até a idade do desmame (n=482) foram incluídas no inverno, primavera e verão. Dados ambientais (Vmax, Vmed, Temperatura, Chill, Ponto de Orvalho, Umidade e Heat Index) foram avaliados em cada fazenda nos momentos de coleta e comparados entre inverno e primavera/verão. Modelo Bayesiano foi utilizado com priors informativos para acurácia UST, Ausc e Hap (sensibilidade (Se) e especificidade (Sp)), e não foram utilizados priors informativos para acurácia de Wisc and Calif. Dados ambientais mostraram que o clima no estado de São Paulo foi predominantemente quente e úmido nas duas estações observadas, e apresentaram diferenças apenas na umidade no meio dia (p=0.03) e tarde (p<0.01), entre primavera/verão e inverno, ponto de orvalho foi maior no meio dia (p=0.01) e tarde (p<0.01) na primavera/verão, não sendo observadas diferenças de temperatura entre as estações do ano. Porcentagem de animais positivos para o teste de Wisc variou entre 40-85%, Calif foi de 8-51%, UTS 0-72%, Ausc 0-32% e Hap 0-100%. A profundidade máxima de consolidação encontrada foi de 4,99cm. A Se (95% credible interval (CI)) e Sp Wisc foi de 97,3% (89,4–99,9) e 52,5% (46,6–58,8). Para Calif Se foi 60,0% (47,6–72,4) e 80,7% (75,8-85,5), TUS apresentou Se 67,7% (57,2–77,8) e 87,8% (83,3–92,1), AUSC Se foi de 50,7% (37,5-67,2) e Sp foi de 99,2% (96,9-1), Hap apresentou Se e Sp de 68,0% (58,7-76,7) e 51,0% (46,1-56,1). Os escores avaliados apresentaram perfis inversos de acurácia, Wisc com alta Se e baixa Sp, e Calif com alta Sp e baixa Se, necessitando de adaptação para sua utilização. Nenhum teste específico atingiu níveis de Se e Sp para diagnosticar a presença de broncopneumonia em bezerras de forma exclusiva, sendo assim a utilização da combinação dos escores que possam ser utilizados como triagem associados a ultrassonografia podem reduzir os diagnósticos realizados de maneira inadequada.

Palavras-chave: DRB. Ultrassonografia. Escores. Haptoglobina. Acurácia.

ABSTRACT

DECARIS, N. **Bayesian Framework of the sensitivity and specificity of five pneumonia detection methods in pre weaning calves.** 64 p. Dissertação (Mestrado em Ciências) -Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2021.

Calves' bronchopneumonia is a multifactorial disease, which causes short- and long-term economic losses, being early detection a crucial factor in relation to therapeutical response, disease severity and mortality. Due these factors clinical scores have been developed in North America for screening sick animals at farm level, as well as the use of ultrasonographic scanning to detect consolidation lesions in lung parenchyma. Over the environmental conditions and raising system, the diagnostic methods developed at North America has been applied in subtropical and tropical countries. Due theses facts, this research evaluated the accuracy of five methods to diagnose bronchopneumonia in Holstein dairy calves at different raising system in subtropical conditions. The tests used to diagnose bronchopneumonia were thoracic ultrasound (TUS, positive if consolidation depth ≥1cm), auscultation (Ausc, positive in presence of crackles, wheezes and pulmonary silence areas), Wisconsin score (Wisc, positive if ≥4), Californian score (Calif, positive if ≥5) and serum haptoglobin concentration (Hap, positive if ≥15 mg/dL) for BRD diagnosis. Heifers with 30 days to weaning age (n= 482) of 17 commercial farms in São Paulo state were enrolled at winter, spring and summer seasons. Environmental data (Vmax, Vmed, Temperature, Chill, Dew Point, Humidity, Heat Index) was assessed in each farm in sampling and was compared between winter and spring/summer. Bayesian latent class models were used with informative priors for TUS, AUSC and Hap accuracy (sensitivity (Se) and specificity (Sp)) and non-informative priors for Wisc and Calif accuracies. Environmental data showed that São Paulo climate was predominantly hot and humid in the two seasons, and differs only at humidity levels presented difference in the middle day ($p=0.03$) and afternoon ($p<0.01$) between spring/summer and winter season, and dew point was higher in middle day ($p=0.01$) and afternoon ($p<0.01$) at summer/spring, no differences was observed at temperature levels between seasons. Percentual of positives calves to bronchopneumonia for Wisc score vary from 40 to 80%, Calif escore was 8-51%, TUS 0-72%, Ausc 0-32% and Hap 0-100%. Maximum consolidation depth obtained was

4,99cm. The Se (95% credible interval (CI)) and Sp Wisc were 97.3% (89.4–99.9) and 52.5% (46.6–58.8), For Calif Se was 60.0% (47.6–72.4) and 80.7% (75.8-85.5), TUS presented Se 67.7% (57.2–77.8) and 87.8% (83.3–92.1), AUSC Se was 50.7% (37.5-67.2) and Sp was 99.2% (96.9-1), Hap presented Se and Sp of 68.0% (58.7-76.7) and 51.0% (46.1-56.1). Evaluated scores presented inverse accuracy levels, Wisc with high Se and low Sp, and Calif with high Sp and low Se, needing to be adapted by their utilization. No specif test achieved Se and Sp levels to diagnose alone the bronchopneumonia in calves, therefore the use of scores for screening BRD associated to TUS can reduce the inadequate diagnosis.

Keywords: BRD. Ultrasonography. Scores. Haptoglobin. Accuracy.

SUMMARY

1. GENERAL INTRODUCTION

Brazilian has the largest commercial herd in the world, represented by 214,7 million heads of cattle, which produces 10.2 million tons of meat, exporting 1,5 million of tons, and 35.9 million tons of milk per year (IBGE, 2020). In this scenario the death or delay on growth of calves means considerable losses, since the costs with raising dairy calves are the second largest production costs, behind only to feeding costs, being estimated in $2,510 per animal from birth to production stage (AKINS; HAGEDORN, 2016).

At calf level the most common diseases responsible for deaths reported by farms monitored by National Animal Health Monitoring System, are diarrhea with 56,4% of deaths and respiratory problems, such as BRD, cause of 24% of deaths, weaned heifers presents 58,9% of deaths caused by respiratory problems (USDA, 2014). Brazilian report shows an incidence of BRD equal to 24.8% in preweaned calves, and observed mortality of 6.3% (AZEVEDO at al., 2020). Instead higher mortality levels, the occurrence of BRD leads reduced growth and average daily gain, calves with BRD where 11kg lighter at 6 months of age compared to health calves (DUNN et al., 2018), increase the mean age of first calving, calves that received pneumonia treatment presented at mean 9 days more for the first calving than not treated animals (CLOSS JR; DECHOW, 2017), and reduction on milk production, animals that presented lung consolidation resulted in a 525 kg decrease in first-lactation 305-d milk production (STANTON et al., 2012).

Annual losses associated to BRD was estimated by Van der Fels-Klers (2001) around €31.20 (€18.4 to €57.1) by heifer sick at farm. Dubrovsky et al, (2020) estimated associated loss due to BRD per treatment of preweaned calves, including costs with medication, labor and reduction in the calves average daily gain. At this report calves first case of BRD costed $36.43 without using anti-inflammatory medications (AIM) or $36.95 including use of AIM, 14.7% of calves were diagnosed with a second BRD case, generating costs $31.14 or $31.69, with or without use of AIM, respectively. Third case of BRD were diagnosed at 1.7% of calves, costing to the farm $31.57 when not using AIM in treatment protocol or $32.10 using anti-inflammatory drugs associated, so total costs of BRD can vary from $36.43 to $41.54 depending on the treatment protocol and times of calves were affected with BRD. Overton, (2020) estimated costs associated to BRD per case of $ 252 or $282, depending if consider the estimated of difference on milk production at the first lactation, the model reflects losses not estimated in previous reports when heifers are culled for beef market.

The modern clinical view obliges the veterinarian to work with the best possible cost-benefit ratio, in this way, accurate and early diagnosis, will provide less expenses with the treatment of this animal for the producer, and there will also be less sequelae and rates of mortality (GONÇALVES et al., 2001).

But BRD represents a challenge to diagnosis due to the unspecific and varying clinical signs and multifactorial etiology. To supply these requirements several researches are being developed to reach efficient in vivo diagnosis methods. The most common diagnosis method performed by veterinarians is pulmonary auscultation, but efficiency of the method is related to the experience of the operator (GONÇALVES et al., 2001). Universities at USA developed clinical scores to diagnosis BRD observing clinical signs such as temperature, cough, ocular and nasal discharge, position of head and ears, respiratory type (MCGUIRK, 2008; LOVE et al., 2014). The methods, which the major advantage is that they are an easy tool for producers, presented approximated sensibility of 72% and approximated specificity of 90% in a previous report (LOVE et al., 2016), but the methods could be influenced by environmental factors such as high temperatures and heat stress, increasing respiratory rate and rectal temperature (ROLAND et al., 2016). Recently thoracic ultrasonography has been receiving a lot of attention as an easy and fast method to perform at the farm (OLLIVETT; BUCZINSKI, 2016). Bacterial and occasionally viral agents result in lesions at pulmonary lobes, modifying the lung tissue density, that alters US image, making possible detection of pulmonary lesions (OLLIVETT et al., 2015). The exam presented sensibility of 94% and specificity of 100% (OLLIVETT et al., 2015).

There is no gold standard available to diagnosis or monitor BRD, for this reason veterinarians and producers need to know the accuracy with the influence factors, such as higher temperatures or concomitant diseases at Brazil, that could affect the performance of these methods when monitoring the disease at herd level for make the best choice considering the advantages and disadvantages for each method.

2. LITERATURE REVIEW

2.1. DEFINITION

Bovine respiratory disease (BRD) is a generical term with uncertain diagnosis, also called acute undifferentiated bovine respiratory disease, that englobe diseases affecting upper respiratory tract, such as rhinotracheitis, rhinitis and enzootic nasal granuloma, than lower respiratory tract as bronchopneumonia, and interstitial pneumonia (CONSTABLE et al., 2017a). Bronchopneumonia is characterized by the invasion of pathogens to the lung and pulmonary tree (WOOLUMS, 2019).

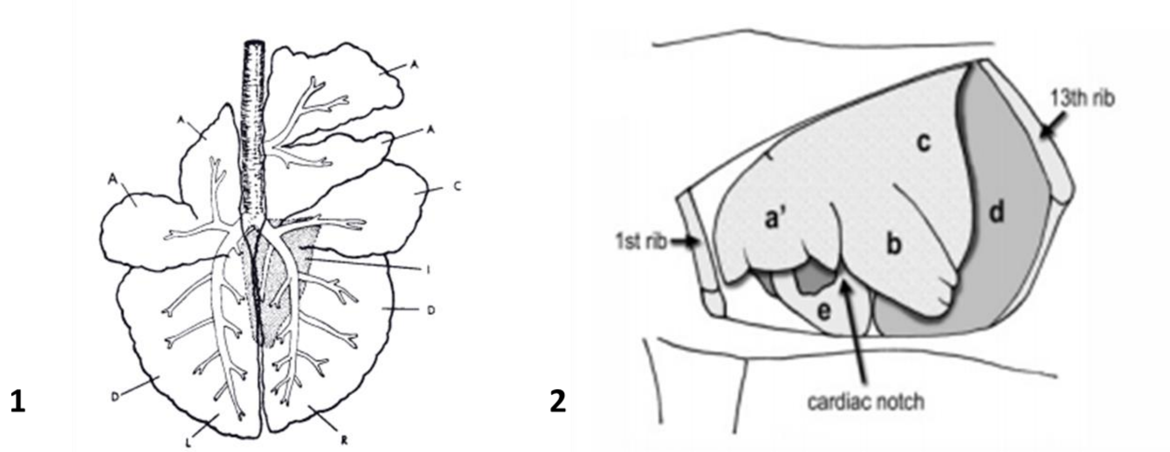
2.2. ETIOLOGY OF BRD

BRD is an multifactorial disease, resulting from the interaction of the host, the microbiological agents and the environmental and management risk factors (BUCZINSKI; BORRIS; DUBUC, 2017; CONSTABLE et al., 2017a). The three pillars that cause pneumonia in cattle are described below.

2.2.1. HOST-RISK FACTORS

Respiratory system is divided in upper respiratory tract that comprehend nasal cavity, pharynx and larynx, and lower respiratory tract, trachea and lung parenchyma. Cattle presents the left lung is divided into cranial (apical, closely to the pleural apices) and caudal (diaphragmatic, ventrally to the pericardium) lobes, with the cranial lobe having a further division into cranial and caudal, and right lungs is divided into four lobes: cranial and caudal lobes as on the left, plus a middle lobe between these and a ventrocaudal accessory lobe near the midline (JACKSON; COCKCROFT, 2007; FRANDSON; WILKE; FAILS, 2009).

Figure 1 – Anatomical scheme for bovine lung (1), and disposition of bovine the left lung in a cutaway view of the thorax (2)



(1) A – cranial lobes; C – middle lobes; D – caudal lobes; I - accessory lobe; L – left; R - right

(2) a’ – cranial part of cranial lobe; b – caudal part of cranial lobe; c – caudal lobe; d – diaphragm; e- heart.

Source: Adapted from (FRANDSON; WILKE; FAILS, 2009)

Bovine has some anatomical particularities at respiratory system that can predispose to respiratory diseases, such as high percentual of interstitial tissue, causing less complacency and higher resistance of pulmonary tissue. The rigid chest cage makes the diaphragm responsible for the ventilation, narrow airways and lung compartmentalization are responsible to less effective gas exchange (BATISTA, 2015). Cattle have long tracheobronchial tree comparing to others species, resulting in higher dead space volume leading to reduce the amount of fresh oxygen delivered to lung, increase the risk of alveolar hypoventilation with partial obstruction, increase the surface area to deposition of particles and increased transit time of inhaled vapors, gases, and particulate matter (ACKERMANN; DERSCHEID; ROTH, 2010). Higher basal ventilatory activity, resulting in remain inspirated air that became more contaminated progressively, higher degree of compartmentalization predisposing to hypoxia in the airways resulting in lower mucociliar and phagocytic activity. Cattle presents low numbers of alveolar macrophages, and low level of bioactivity of lysozyme, leading to impaired pulmonary clearance comparing to other species (FLÖCK, 2004).

2.2.2. ENVIRONMENTAL AND MANAGEMENT RISK FACTORS

Instead these natural factors some management factors are associated to development of BRD, such as failure of passive transfer (FPT) (DONKERSGOED et al., 1993; FURMAN-FRATCZAK; RZASA; STEFANIAK, 2011; WINDEYER et al., 2014), inadequate vaccination protocols (GORDEN; PLUMMER, 2010), diet restriction, such as reduced milk feeding, resulting in a reduction of the immune response (KHAN; WEARY; VON KEYSERLINGK, 2011), and environmental factors as collective pens with a higher number of animals (SVENSSON; LIBERG, 2006), direct contact with older heifers and cows (LUNDBORG; SVENSSON; OLTENACU, 2005), poor quality of bed and air (LAGO et al., 2006), inadequate ventilation, stress factors such as caused by transportation, dehorning or weaning (TAYLOR et al., 2010). Risk factors include also feeding calves with mastitic colostrum, occurrence of diarrhea , leave calves with the dam more than 24 hours, automatic feeders with groups with 6 to 30 calves, larger herd size, more than 8 week age range in the same pen, sharing housing with cows (WOOLUMS, 2019).

Buczinski et al (2017) observed most prevalence of ultrasonographic consolidation (≥3 cm of depth) at winter comparing to summer, 15% and 8%, respectively, and in farms that housed animals in groups before weaning (19.5%), comparing to individual housing (6.7%).

Dubrovsky et al (2019), studies some management factors that impacts on hazard to development BRD in dairy calves from California. They founded that the hutches made of a combination of metal and wood provides more risk than hutches made only of wood or plastic (24%). At feeding level, giving more than 3.8 L of milk daily to calves under 21 days of age decreased risk by 92%, and feeding calves with fed only saleable or waste milk reduces 46% risk than milk replacer supplemented calves. Vaccination of dams with modified live vaccine or killed vaccine reduces risk 67% and 15%, respectively. More number of beds changing in maternity pen decreased BRD risk.

2.2.3. MICROBIOLOGICAL AGENTS

The risk factors contribute to the infection of lower airways of the pathogens such as Bovine Respiratory Syncytial Virus (BRSV), Bovine Herpes Virus (BoHV), Bovine Parainfluenza Virus (BPIV-3), Bovine Viral Diarrhea Virus (BVDV), Bovine Corona Virus (BCoV) (FULTON, 2009). The viral pathogens are associated to lead damages to upper and lower airways, individually or in combination, that increase opportunity for secondary bacterial infections due impaired innate immune response. BVDV can harm the function or destroys alveolar macrophages immunosuppressing, BRSV infections in the most cases are asymptomatic, but depresses phagocytosis and opsonization by alveolar macrophages, and infects the epithelial cells inducing loss or necrosis of cilia or necrosis leading a reduced mucociliary clearance, that provides an ideal environment for bacterial colonization, such as occurs in infections of BoHV-1 and PI-3 (GRIFFIN et al., 2010).

Histophilus somni and *Mycoplasma bovis* are frequently observed (SNOWDER et al., 2006; FULTON, 2009; GRISSETT; WHITE; LARSON, 2015). Bacterial agents *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* are natural hosts of respiratory tract but became opportunistic colonizers after the viral infection, whereas *Mycoplasma bovis* can be primary pathogen or co-infection in the disease (GRISSETT; WHITE; LARSON, 2015). Bacterial infection leads to a neutrophilic infiltrate in the bronchial, bronchiolar, and alveolar compartments of the lung, generating, typically, a distribution that starts in the cranial lobe or right middle lobe moving forward in caudal direction, presenting quickly damage, only after 2 hours of bacterial experimental challenge (OLLIVETT, 2014).

Brazilian report isolates the microorganisms of 21 calves located in a farm that presented BRD outbreak, performed PCR in bronchoalveolar lavage samples from asymptomatic (n = 6) and symptomatic (n = 15) calves. Data shows the detection of Pasteurella *multocida* in 42.85 % (9/21) of BALF samples, followed by BRSV detection in 46.6 % (8/21), the major agents envolved in BRD in this study. Mycoplasma bovis and BCoV were present in 33.3 % (7/21), 28.6% (6/21) of BVDV, and 19 % (4/21) of H. somni. Majority of infections were mixed infections (72.2 %) than single infections (27.7 %), and more than a half of mixed infections were occasioned by viruses and bacteria , showing the synergism of these two type of pathogens in BRD infections (OLIVEIRA et al., 2020).

2.3. CLINICAL FINDINGS

Clinical findings associated with pneumonia might be vary as the stage of the disease. In general, shallow breathing, dyspnea, polypnea and coughing are the most common sign in pneumonia cases (CONSTABLE et al., 2017a). Calves might present anorexia, depression, tachycardia, dyspnea, breath sounds are loud and harsh, with increased of intensity in heart sounds, when occurs secondary bacterial pneumonia the toxemia are usually more severe (CONSTABLE et al., 2017a). Depression, fever, and other signs of sepsis (WOOLUMS, 2019), nasal discharge are related to local inflammation, but also to bronchial and pulmonary secretion, dyspnea, dullness at percussion exam, and abnormal lungs sounds due to increase of interstitial inflammatory fluid (GONÇALVES et al., 2001).

2.4. BRD DIAGNOSIS TOOLS

Classical diagnosis is obtained by a clinical exam to evaluate the respiratory system, and are composed by an evaluation of respiratory movements, observing not only the respiratory rate but also respiratory type, normal respiratory type is cost abdominal, and rhythm, with a relation inspiration inhalation:exhalation 1 to 1.2. Larynx and pharynx can be evaluated by external inspection and internal with laryngoscope, and evaluated by palpation of the region. Trachea can be palped and inspected externally, tracheal wash can be performed to mycological, bacteriological and serological tests. Lungs and pleura are examined by observing respiratory movements, acoustic percussion, at horizontal direction in the first moment, stablishing caudal lung limits, and at vertical, stablishing changes at normal pulmonary resonance that should be resonant, and evaluating the sensibility, with a help of a heavy hammer, and auscultation of pulmonary areas (STÖBER; DIRKSEN; GRÜNDER, 1992).

Pulmonary auscultation needs to be performed initiating at trachea going to pulmonary area, in each pulmonary area evaluated by auscultation 1 to 2 respiratory movements need to be auscultated, to intensify pathological sounds, temporary breathing interruption could be performed. Normal breath sounds are formed by air turbulence in airways ramifications. Pathological respiratory sounds detected in BRD cases could be silence areas, caused consolidated areas without airflow, crackles, caused by intense deviation of airflow by obstruction by secretion and exudate, and wheezes, formed by airflow acceleration caused by increased pulmonary density or airway stricture (STÖBER; DIRKSEN; GRÜNDER, 1992).

Gonçalves et al (2001), assed 48 calves to associates the clinical signs presented by calves and the severity of BRD. In this research, cough and positive cough reflex such as increase of rectal temperature and heart rate, disorders at tracheobronchial sounds, instead to be related to diagnosis not discriminate the process intensity. Respiratory rhythm, dyspnea, presence of dullness at percussion, presence of silence areas, crackles or wheezes at auscultation defined severity at calf's bronchopneumonia, due to manifestation at mostly BRD severe cases.

Buczinski et al. (2014a) assed 136 animals to evaluate the accuracy of auscultation, comparing to the ultrasound was 3 to 17% of sensibility and 97.3 to 100% of sensitivity. Andrade (2017) using thoracic ultrasound as gold standard to define BRD positivity found 30% and 94% sensibility and specificity, respectively for the auscultation.

Presence of clinical signs leads to researches develops clinical scores to assess and evaluate clinical signs presented by calves affected by BRD. McGuirk (2008), developed Calf Health Scoring Criteria, or Wiscosin Score, the system attributes punctuation to five clinical sigs as severity of the signs, as presented in figure 2. Each clinical signs can receive the punctuation between 0 (normal), 1 (mild abnormal), 2 (moderate abnormal), 3 (severally abnormal), the observed clinical signs are rectal temperature, nasal discharge, ocular discharge, cough (spontaneous or induced) and position of ears or head tilt, however, when ear position and ocular discharge are both abnormal, only the higher value is included in the score. Calves presenting sum ≥4 are classified as positive for BRD, based on cytology and culture of bronchoalveolar lavage fluid comparing to the score in unpublished data. Each animal evaluation in this score can be performed in less than 2 minutes.

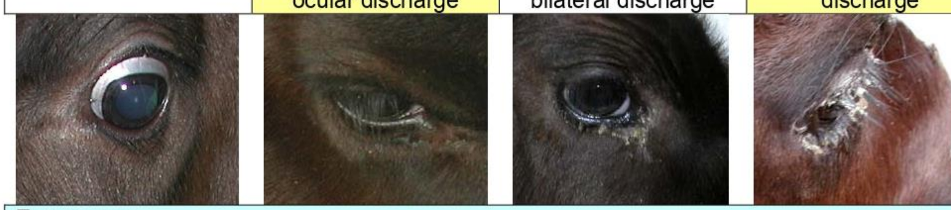
Figure 2 – Bovine respiratory disease (BRD) score system for preweaning dairy calves developed in Wisconsin.

SCHOOL OF VETERINARY MEDICINE
University of Wisconsin-Madison

Calf Health Scoring Criteria

0	1	2	3
Rectal temperature			
100-100.9	101-101.9	102-102.9	≥103
Cough			
None	Induce single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous coughs
Nasal discharge			
Normal serous discharge	Small amount of unilateral cloudy discharge	Bilateral, cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge

0	1	2	3
Eye scores			
Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge



0	1	2	3
Ear scores			
Normal	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop

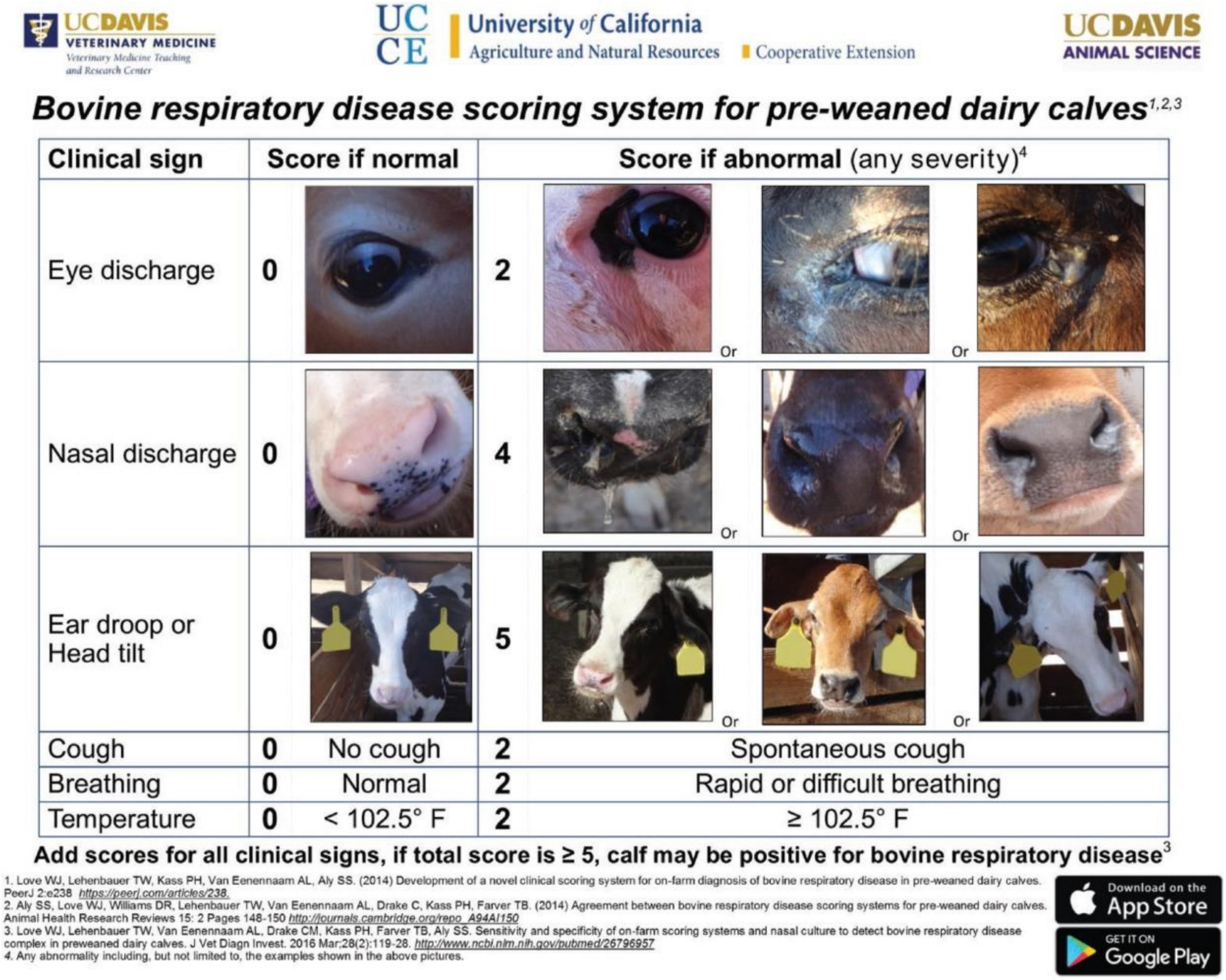
100 – 100.9 ºF = 37 – 38.2ºC
101 – 101.9 ºF = 38.3 – 38.8ºC
102 –102.9 ºF = 38.9 – 39.3ºC
≥ 103 ºF = ≥ 39.4ºC
Source: (MCGUIRK; PEEK, 2014)

Buczinski et al (2014b), observed sensitivity of 55% and specificity of 58% to Wisc score to detect BRD positive calves, using consolidation ≥1cm at thoracic ultrasound as

BRD case definition. Buczinski et al (2015) estimated using a Bayesian approach sensitivity and specificity for Wisc score of 62,4% and 74,1%, respectively.

Love et al (2014) developed another score system, BRD3 (Bovine Respiratory Disease) Scoring System, Californian Score, based at dichotomous observation of nasal discharge, ocular discharge, spontaneous cough, head tilt and ear position, breathing type and rectal temperature, each clinical sign has a value in the score, summarized in figure 3. Animals presenting sum of punctuations more than 5 are classified as BRD positive. The score elaboration used as case definition for BRD: positive PCR for respiratory viruses, bacterial culture positive for aerobial pathogens with Wisc Score ≥4 or positive bacterial culture for *Mycoplasma bovis* with Wisc Score ≥4. The dichotomous system was to consider easier to do at farm level.

Figure 3 – Bovine respiratory disease (BRD) score system for preweaning dairy calves. developed in California.



Clinical sign	Score if normal		Score if abnormal (any severity)[4]	
Eye discharge	0		2	
Nasal discharge	0		4	
Ear droop or Head tilt	0		5	
Cough	0	No cough	2	Spontaneous cough
Breathing	0	Normal	2	Rapid or difficult breathing
Temperature	0	< 102.5° F	2	≥ 102.5° F

Add scores for all clinical signs, if total score is ≥ 5, calf may be positive for bovine respiratory disease[3]

1. Love WJ, Lehenbauer TW, Kass PH, Van Eenennaam AL, Aly SS. (2014) Development of a novel clinical scoring system for on-farm diagnosis of bovine respiratory disease in pre-weaned dairy calves. PeerJ 2:e238 https://peerj.com/articles/238.
2. Aly SS, Love WJ, Williams DR, Lehenbauer TW, Van Eenennaam AL, Drake C, Kass PH, Farver TB. (2014) Agreement between bovine respiratory disease scoring systems for pre-weaned dairy calves. Animal Health Research Reviews 15: 2 Pages 148-150 http://journals.cambridge.org/repo_A94AI150
3. Love WJ, Lehenbauer TW, Van Eenennaam AL, Drake CM, Kass PH, Farver TB, Aly SS. Sensitivity and specificity of on-farm scoring systems and nasal culture to detect bovine respiratory disease complex in preweaned dairy calves. J Vet Diagn Invest. 2016 Mar;28(2):119-28. http://www.ncbi.nlm.nih.gov/pubmed/26796957
4. Any abnormality including, but not limited to, the examples shown in the above pictures.

102.5°F = 39.2°C.
Source: (LOUIE et al., 2018)

Concordance between Wisc and Calif scores was assessed by Aly et al, (2014) in 100 California dairy calves, and obtained a kappa coefficient of 0.85 between scores, an excellent concordance. Love et al (2016) assessed 536 pre weaned calves in California dairies, case definition was realized by thoracic auscultation and/or thoracic ultrasound, resulting in 315 controls and 221 cases, which 86 were randomly selected and 135 were apparently ill, sensitivity and specificity for Wisc score was 46% and 91.2%, and for Caif score was 46,8% and 91,2% respectively.

Complementary exams also can be performed to diagnosis BRD. Masseau et al (2008), demonstrated in retrospective study in 42 adult animals that sensitivity of radiographic exam is 94%, but specificity is 50% comparing to post-mortem lesions. Computerized tomography was evaluated by Lubbers et al (2007), presenting higher correlation to exam and consolidation observed at necropsy (r=0.97). But the both techniques do not have application in farm reality, due to high number of animals in higher production farms, physical restrictions of the equipment, high costs and exposition to radiation (OLLIVETT, 2014).

Ultrasound became highlights in the last years by the applicability at the farm level, it is a portable device and commonly already available in the farms. The pulmonary evaluation can detect and characterize pleural effusion, superficial lung lesions, consolidation, atelectasis, pneumothorax, and in some cases can be more precise than radiography (BABKINE; BLOND, 2009), is easier to do at farm, instead performed by an equip trained to interpret the exam. Bacterial an occasionally viral agents of BRD leads to alterations in pulmonary lobe that changes their density, causing alterations at ultrasound lung image since artefacts with reverberation until to a homogeny hyperechoic structure same as liver ultrasound image (lung consolidation) (OLLIVETT et al., 2015; BUCZINSKI; BORRIS; DUBUC, 2017). Experimental infections caused by *Mannheimmia haemolytica* leads to lung consolidation after 2 hours infection, so it is common that consolidation appears even before the BRD clinical signs, and animals with chronic disease could not present clinical signs associated to the disease (FORT et al., 2015). The TUS presented 94% of sensibility and 100% of specificity comparing the lesions post mortem and histopathologic exam in animal lungs (OLLIVETT et al., 2015). Auscultation associated to the TUS interpreted in parallel increased the sensibility and reduced the specificity of DRB comparing to methods separated (LOVE et al., 2016).

3. OBJECTIVE

BRD is one of the most important disease in calf rearing, accurate diagnosis and correct treatment is a key point in the disease management. For this reason, the main objective of this research is determining the accuracy of thoracic auscultation, thoracic ultrasound and two available scores developed in USA (Californian and Wisconsin Scores) to diagnosis BRD on pre weaned calves in Brazilian subtropical conditions.

The hypothesis is that the geographic and climate particularities can influence on the accuracy of the scores developed in the USA on a different humidity and temperatures index. So, the general aim was to evaluate the scores performance under subtropical environmental conditions, and also investigated the accuracy of the thoracic ultrasound, auscultation, haptoglobin in calves of commercial farms in pre weaning period.

4 DIAGNOSTIC ACCURACY OF WISCOSIN AND CALIFORNIAN SCORING SYSTEMS TO DETECT BRONCHOPNEUMONIA IN DAIRY CALVES RAISED IN TROPICAL COUNTRY

4.1 INTRODUCTION

Bovine respiratory disease complex is a multifactorial disease due to the interaction between host, microbiological agents, including viruses and bacteria, and environmental factors (GRISSETT; WHITE; LARSON, 2015). This disease affected 12% of preweaned dairy calves, and it is responsible for 24% of preweaning deaths in the USA dairy farms (USDA, 2014). In a large research, it was reported that 21.9% of calves were treated at least once for BRD, and 19.4% of these calves were treated more than once (WINDEYER et al., 2014). Almost all calves manifesting BRD (94.8%) have been treated with antimicrobials, especially macrolides and florfenicol (USDA, 2014). BRD is also a concern in a tropical country such as Brazil, and the epidemiological information is very similar to North American data. The Brazilian BRD prevalence, observing 24,684 dairy preweaned calves, was 24.8%, and it is a leading cause of preweaning mortality in dairy (equal 6.3%) (Azevedo, 2020).

Overall cost of calfhood BRD is reflected in both immediate cost of treating the disease as well as lifetime decrease in production and the decrease of cow’s longevity. In general, estimated cost of BRD per affected case was $42.15, considering different treatment protocols, use of anti-inflammatory drugs, costs with energy, labor, in various management conditions at California dairy production scenario (DUBROVSKY et al., 2020). Thoracic ultrasonography (**TUS**) has been reported as a rapid tool to be performed by veterinarians at farm and identify lung abnormalities as lung consolidation in preweaned dairy calves (OLLIVETT; BUCZINSKI, 2016). Lung consolidation has been associated with decreased average daily gain (STANTON et al., 2012), increased 30-day mortality (BUCZINSKI; L OLLIVETT; DENDUKURI, 2015), increased risk of being culled before the 1st calving (ADAMS; BUCZINSKI, 2016), and lower reproductive performance (TEIXEIRA; MCART; BICALHO, 2017). For long-term production outcomes, dairy heifers manifesting lung consolidation in the first 8 weeks of life produced 525 kg less milk in the first lactation (DUNN et al., 2018). The reduction in BRD disease may be also associated with additional cost savings and an improvement in calf welfare and herd life (DUBROVSKY et al., 2019).

The accurate diagnosis of bronchopneumonia, discerning between upper and lower respiratory tract disease, is a challenge due to the lack of sensitive and specific clinical signs. Actually, there is no gold standard to diagnosis bronchopneumonia, and veterinarians have been used the clinical examination of the respiratory tract, especially thoracic auscultation (AUSC) to detect abnormal lung sounds, and complementary exams as radiography and ultrasound depends on the availability. The screening of calves manifesting clinical signs of BRD on-farm by producers and workers have been done based on the set of visual signs of respiratory disease grouped in different scoring system that are intended to be used various people (MCGUIRK, 2008; LOVE et al., 2014)

Recent studies have compared the accuracy of different diagnosis methods to detect bronchopneumonia. Buczinski et al (BUCZINSKI; MÉNARD; TIMSIT, 2016) evaluated the thoracic ultrasound (TUS) and auscultation in the detection of lower respiratory tract infection, and these authors reported that AUSC was a sensitive method (72.9%; 95% Bayesian credible interval [BCI]: 50.1–96.4%), but not specific (53.3%; 95% BCI: 43.3–64.0%). On the other hand, TUS was more specific (92.9%; 95% BCI: 86.5–97.1%), with a similar sensitivity of AUSC (76.5%; 95% BCI: 60.2–88.8%). Bayesian estimation detected a median sensitivity and specificity (62.4% and 74.1%, respectively) for the Wisconsin Calf Health Scoring proposed by McGuirk (2008) (BUCZINSKI; L OLLIVETT; DENDUKURI, 2015).

Recent researches of our group presented a high outcome for positivity using Wisconsin score system to detect BRD, with 73% (438/600) of positivity to BRD at score evaluation level, which after a respiratory clinical exam was interpreted as false positive results (GOMES, 2021). Tropical conditions, as Brazil, have a high temperature and humidity level, resulting in a high stress index, increasing respiratory rhythm and rectal temperature (KOVÁCS et al., 2020), that could induce confusions factors in the parameters of Wisconsin and Californian BRD scores. Recently, Maier et al, (2019) published a BRD score system for weaned animals, and shown that the scoring in post-weaned calves should be adjusted for specific weather condition, assessed as diurnal range of temperature.

For this reason, our hypothesis was that the geographic and climate particularities can influence the accuracy of the scores developed in the USA on a different humidity and temperatures index. Comparing the sensitivity and specificity of each test, can be helpful for veterinarian at subtropical climates areas the best test option to make the diagnosis of BRD. The main objective was to determine the accuracy of thoracic auscultation, thoracic ultrasound and two available scores to diagnosis BRD on preweaned calves in subtropical conditions.

4.2 MATERIALS AND METHODS

This study was approved by the School of Veterinary Medicine and Animal Science of the University of Sao Paulo Animal Care and Use Committee (Protocol number: 5689310119).

4.2.1 Assessment of Herd and Calves

This prospective study was conducted in winter (July up to September 21th 2020), spring (September 22nd up to December 21st 2020) and the early of summer (December 22nd up to January 31st 2021).

Seventeen herds located in São Paulo city - Brazil, were visited at least once by the research team for data collection. Young Holstein dairy heifers in the pre-weaning phase from 30 days of life that did not received vaccination for BRD in the previous 15 days before the ultrasound evaluation were screened. Recent treatment for BRD were not used as an exclusion criterion. Farms with less than 50 eligible calves, all eligible preweaned Holstein heifers were included in the study only once. When were more than 50 eligible calves, they were randomly selected by excluding 3 pens and selecting the next 1 pen. The screening process resulted in the inclusion of 482 young heifers aged from 30 up to 127 days of life (median of 57 days).

4.2.2 Calf Rearing Practices

Calves were maintained with regular routine of farms regarding to the diet, housing and health management. Specific informations concerning calf management during the preweaning period, based on BRD questionnaire risk developed by Love et al, (2016) and Aly et al, (2020) was collected during the farm visit. Descriptive information of the average numbers of milking cows, colostrum source, evaluation of quality, quantity and quickness of colostrum management, vaccination protocols for preweaning calves, weaning age was collected.

The descriptive presentation of calf management on these farms is shown in the table 1, location of farms are represented at figure 4.

Table 1 – Description of farm location, number of visits and calf management of selected herds.

N. FARM	Nº OF VISITS	MILKING COWS (N)	COLOSTRUM SOURCE	ADOPTION OF 3Q'S COLOSTRUM MANAGEMENT[1]	MILK SOURCE	MINIMUM AMOUNT OF 6L MILK FEEDING	HOUSING	VACCINATION[2]	WEANING AGE (DAYS)
1	1	113	Fresh and freezed	Yes	Fresh milk	No	Individual	Yes 180 d SC	60-90
2	1	150	Fresh and freezed	Yes	Waste milk	Yes	Grouped	Yes 90 -120 d SC	90
3	2	170	Fresh	No	Waste milk and milk replacer	No	Grouped	No	140
4	3	314	Fresh and freezed	Yes	Milk replacer	Yes	Individual until 30 days, grouped until weaning	Yes 0-10 and 60 d IN 90 to 120 d SC	75
5	1	590	Fresh and Freezed	Yes	Acidified waste milk	Yes	Grouped	Yes 1 and 60 d IN 90 to 120 d SC	90-100
6	1	840	Fresh and freezed	Yes	Waste Milk	Yes	Grouped	Yes 1 and 60 d IN 90 to 120 d SC	80-90
7	1	45	Fresh and freezed	Yes	Waste milk	Yes	Grouped	Yes 90 to 120 d SC	90
8	2	2076	Fresh, freezed and replacer	Yes	Waste milk and milk replacer	Yes	Individual	Yes	80
9	2	590	Fresh and freezed	Yes	Waste milk and milk replacer	Yes	Individual	Yes 1 and 60 d IN 90 to 120 d SC	80-90
10	1	120	Fresh	No	Waste milk	No	Pairs	No	100
11	1	300	Fresh and freezed	Yes	Milk replacer	Yes	Individual	No	60
12	1	180	Fresh	No	Waste milk	No	Individual	Yes 1 and 60 d IN 90 to 120 d SC	80-90
13	1	85	Fresh and freezed	Yes	Waste milk	Yes	Individual until 15 days, grouped until weaning	Yes 90 to 120 d SC	80-90

14	1	118	Fresh and replacer	No	Waste milk and milk replacer	Yes	Grouped	Yes 60 d SC	90
15	4	430	Fresh, freezed and replacer	Yes	Milk replacer	Yes	Grouped	Yes 90 d SC	60-70
16	3	292	Freezed	Yes	Milk replacer	Yes	Grouped	Yes	90-120
17	1	170	Fresh	Yes	High SCC	No	Individual until 40 days, grouped to weaning	No	90

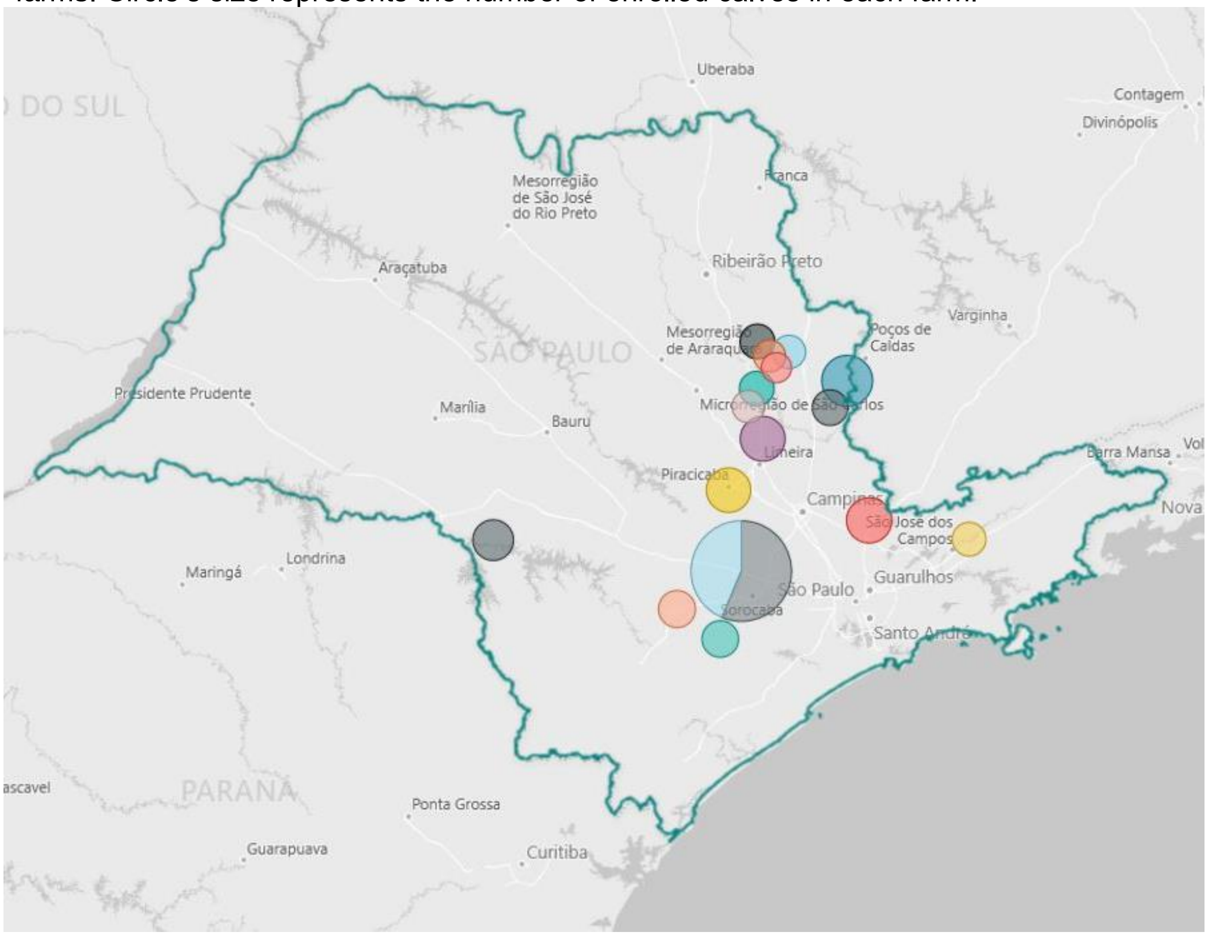
Source: DECARIS, 2021

[1] Measurment of colostrum quality, at least 10% of BW in the first offering of colostrum, colostrum intake at the fisrt 6 hours of life.

[2] Vaccination against the major pathogens to BRD

IN: Intranasal; SC: Subcutaneus

Figure 4 – São Paulo state map, identifying the location of selected commercial dairy's farms. Circle's size represents the number of enrolled calves in each farm.



Source: DECARIS, 2021

4.2.3 Assessment of Air Draft, Temperature, Hygrometry

Ambiental profile was assessed using a portative anemometer (Kestrel 3000, Nielsen-Kellerman, Boothwyn, PA) including wind speed (0.4–40m/s; accuracy ± 3%), temperature (-29.0°C to +70.0°C; accuracy ± 1ºC) and relative hygrometry (5%-95%; accuracy ± 3%). The sampled period was 2 minutes of duration, 20 to 30 cm from the ground of the pens, in individual pens was recorded at the pen localized on the middle of the facility, when calves were raised in group, the middle of the installation was the site of sampling. Recordings were stored in an Excel sheet for further analysis.

4.2.4 Clinical scores assessment

Calves were evaluated by one specific research technician by using the Wisconsin BRD Score (*Calf Health Scoring Criteria)* (McGuirk, 2008) and Californian BRD Score (*BRD3 Bovine Respiratory Disease scoring system)* (Love et al., 2014). Briefly, the Wisconsin score is based on 5 criteria: nasal discharge, ocular discharge, rectal temperature, cough and ear position, for each parameter the animal could receive score 0 to 3 according to the severity of the symptoms. Animals with parameters sum ≥4 are considered BRD positive with treatment recommendation. The Californian Score is a dichotomous score with nasal discharge, ocular discharge, rectal temperature, ear position, spontaneous cough and abnormal breathing, as the Wisconsin Score. The weight between the different clinical signs is not the same by contrast to Wisconsin score. If the sum ≥5, calves are considered as BRD positive with treatment recommendation.

4.2.5 Thoracic Auscultation

Pulmonary auscultation was performed as described by Stöber et al. (1992) with a Littmann Classic II® stethoscope in the pulmonary area, examining the dorsal, middle and ventral areas, using two breathing cycles for each auscultation point. All the pulmonary area in the both sides was assessed. The presence of any abnormal lung sounds as crackles, wheezes or lung silence areas. We did not detect the absence of pulmonary sounds during the development of this study. These abnormal sounds were recorded as a positive auscultation test, presence of increased laryngotracheal and tracheobronchial sounds was also recorded but considered as negative since considered as subjective finding with low inter-rater reliability (Pardon et al., 2019). The auscultation classification was the same as previously described by Buczinski et al. (2014a).

4.2.6 Thoracic Ultrasound

Bilateral pulmonary ultrasound assessment was realized as Ollivett and Buczinski (2016), screening all pulmonary area from the 10th to 1st intercostal spaces focusing on lung consolidation. The operator was blinded to the others tests when conducting TUS.

Ultrasonography was performed using a Mindray M5 Vet device (Mindray, Shenzhen, China) with a 6.5MHz linear probe directly applied on the unclipped thorax after using 70% isopropyl alcohol on the area to improve the image quality. Presence of lung consolidation, defined as heterogeneous hypoechoic area without the clear line of the pleural surface, was measured with ultrasound caliper function when performing the exam. Consolidation with ≥ 1 cm of depth was considered as relevant and animal was classified as positive in this test (BUCZINSKI; L OLLIVETT; DENDUKURI, 2015). Animals with comet tail artefacts, pleural irregularity and pleural effusion were registered but not considered as abnormal since these ultrasonographic markers are subjective when assessed by different raters (BUCZINSKI et al., 2018a). Operator performing TUS was blinded for scores and clinical exam results.

4.2.7 Haptoglobin

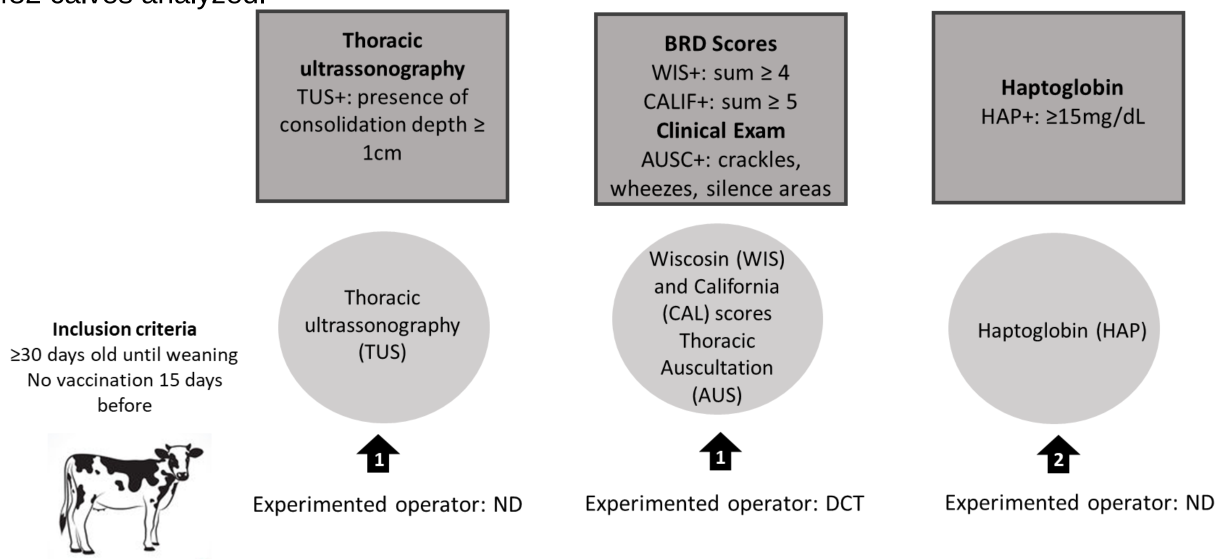
Blood samples were collected for each calf using jugular vein site. The samples were centrifugated at 3000 rpm. Serum was then separated and aliquots stored in duplicate at -80ºC. The concentration of haptoglobin was assessed using a turbidimetric assay according to the procedures described by Ramos et al. (2021). The standard curve was prepared using a serial dilution control serum with a known level. The determination of serum haptoglobin concentration was calculated by interpolation a linear regression of the standard curve for each assay after collecting based on the absorbance value recorded with microplate reader (450 nm). The intra-test coefficient of variation was 7.25% and inter-test coefficient was 8.19%.

In the absence of specific BRD validated threshold for this specific test, haptoglobin concentration ≥ 15.0mg/dL were assigned as BRD positive. This cut-point was a priori selected based on its accuracy to classify BRD calves using previously reported by ther authors using an commercial kit based on Hp-haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin (SVENSSON; LIBERG; HULTGREN, 2007). Haptoglobin concentration <15.0mg/dL were classified as negative results.

4.2.8 Classification of BRD, according to the different diagnosis methods

The classification of animals in positive and negative for each diagnosis methods are shown in the figure 5.

Figure 5 - Screening process to select Holstein young heifers (n= 502) enrolled in the study to perform the thoracic ultrasound (TUS), Bovine Respiratory Disease (BRD) Scores and auscultation (AUSC) during each visit in the commercial farms enrolled in this study, resulting in 482 calves analyzed.



Source: DECARIS, 2021

4.2.9 Statistical analysis

Normality of the ambiental profile, as maximum wind speed (m/s), mean wind speed (m/s), temperature (ºC), chill (ºC), humidity (%), heat index and dew point, was assessed by Shapiro-Wilk test at GraphPad Prism software version 8, parametric data was tested by Paired t-test and non-parametric by Mann-Whitney. Values $p < 0.05$ were considered statistically significant. Descriptive statistics were obtained for the different practices characteristics and calves population. The cross-classification resulting from the combination of the 5 dichotomous tests results were then compiled. A Bayesian latent class model (BLCM) was then applied using a 1 population 5 tests framework following the standard for reporting BLCM (KOSTOULAS et al., 2017). Because the Wisconsin and California scores were based on the same type of biological assessment (eg same clinical signs assessment) these two tests were considered as conditionally dependent with covDp (covariance of Wisconsin and Californian score in cases with active pneumonia) and covDn (covariance of Wisconsin and Californian score in cases without active pneumonia). The likelihood of the 2^5=32 different possible combinations of subpopulation count of tests results profiles (O_j) was then specified using a multinomial distribution:

O_j ~ multinomial (p_j , n) for j=1, 2, …, 32,

where p_j is a vector of probability of the 32 different combinations of tests patterns and n the total number of included in the study calves (n=482).

The specific vector p_j was then described as the probability of observing the 32 individual patterns for tests results (positive (+) or negative (-) based on the prevalence of active BRD in the whole population (π) and tests sensitivities (Se) specificities (Sp) and covariance between the clinical scores (covDp and covDn):

p_1(+,+,+,+,+)= π*Se-Ausc*Se-TUS*Se-Hap*(Se-Wisc*Se-Calif+covDp) + (1- π)*(1-Sp-Ausc)*(1-Sp-TUS)*(1-Sp-Hap)*((1-Sp-Wisc)*(1-Sp-Calif + covDn)

p_2(+,+,+,+,-)= π*Se-Ausc*Se-TUS*Se-Hap*(Se-Wisc*(1-Se-Calif)-covDp) + (1- π)*(1-Sp-Ausc)*(1-Sp-TUS)*(1-Sp-Hap)*((1-Sp-Wisc)*Sp-Calif - covDn)

…

p_{32}(-,-,-,-,-)= π*(1-Se-Ausc)*(1-Se-TUS)*(1-Se-Hap)*((1-Se-Wisc)*(1-Se-Calif)+covDp) + (1- π)*Sp-Ausc*Sp-TUS*Sp-Hap*(Sp-Wisc*Sp-Calif+covDn))

The covariances between the two clinical scores were specifically parametrized as previously described (DENDUKURI; JOSEPH, 2001):

covDp = corr(Wisc, Calif|BRD) ~ U(0, min(Se-Wisc, Se-Calif)- (Se-Wisc* Se-Calif))

covDn = corr(Wisc, Calif|No BRD) ~ U(0, min(Sp-Wisc, Sp-Calif)- (Sp-Wisc* Sp-Calif))

Prior information on test accuracy

Prior information was available for some diagnostic tests used in this study for the main model (Model 1). The prior determination was determined using the best guess and 95^{th} upper and lower limits using the R PriorGen package to determine beta distribution characteristics (Kostoulas, 2018). Auscultation diagnostic criteria used in this study were intended to be specific since based on abnormal sounds auscultation removing subjectivity from bronchial sounds intensity. When using this classification a high specificity but a poor sensitivity is expected (BUCZINSKI et al., 2014b). In this former study using lung lesions as a gold standard, the sensitivity was 5.9% (range from 0 to 16.7%) and specificity 98.7% (range from 97.3 to 100%). The best guess for auscultation sensitivity (Se-Ausc) was set 10% being 95% confident it was lower than 50% which leads to a beta (0.69,3.55) prior. The best guess for auscultation specificity (Sp-Ausc) was 90% being 95% confident the true value was above 50% which leads to a beta (4.9,0.84). For thoracic ultrasound using ≥ 1cm for test positivity, we used the same prior than formerly used from experts' consensus previously (BUCZINSKI; L OLLIVETT; DENDUKURI, 2015). The best guess for ultrasound sensitivity was 85%, being 95% sure it was higher than 70% (beta (23.9, 5.04)). The best guess for ultrasound specificity was 90%, being 95% sure it was higher than 80% (beta (42.57, 5.62)).

For haptoglobin accuracy we used the same priors used in Berman et al (2019) study when using the ≥ 15 mg/dL cutoff obtained from Svensson et al (2007). Briefly the best guess for Se-Hap was 65% being 95% confident that it was above 50% (beta (21,11.8)). The best guess for Sp-Hap was 70% being 95% sure it was above 60% (beta (47.5; 20.9)).

Since the aim of the study was to determine the accuracy of the two clinical scoring systems in tropical condition, non-informative prior (ie uniform distribution from 0 to 1 with beta (1,1)) were selected for both scores sensitivities (Se-Wisc, Se-Calif) and specificities (Sp-Wisc, Sp-Calif). We anticipated that the different scores accuracy differed from what could have been determined in previous US and Canadian studies.

The total number of degrees of freedom was 2^5-1=31 for 5 tests combinations in 1 population. Since a total of 5 test sensitivities and specificities, BRD prevalence (π), covDp and covDn were the 13 unknown parameters to determine, no specific prior informations were required to run the model. For this reason a non-informative model where all accuracy parameters and prevalence were indicated as uniform (beta(1,1)) distribution with all probability from 0 to 1 having the same weight. This non-informative model (Model 2) was used for sensitivity analysis of the results. Both models used the same prior information for covariance parameters.

All models were run using OpenBUGS, R2OpenBUGS package and R software program (STURTZ; LIGGES; GELMAN, 2010; R DEVELOPMENT CORE TEAM, 2020). A total of 30,000 iterations were run and after a 5,000 burn-in iterations, the 25, 000 iterations using 3 different chains starting with different inits were used for estimation of posterior densities. Autocorrelation plots between iterations was visually checked but no specific thinning was required. Convergence was determined using visual assessment of Gelman-Rubin plot and was rapidly obtained in both models.

The posterior densities median and 95% Bayesian credible intervals were then obtained. The deviance information criteria (DIC) were also obtained as an indicator of the model fit. A difference of 5 or more has been previously considered as a indicative of a better fit (ADRION; MANSMANN, 2012).

4 RESULTS

A total of 501 young Holstein heifers were available to include in this study, however 17 calves were excluded due to data missing for haptoglobin or clinical scoring parameters. Then, it was analyzed data from 482 Holstein young heifers distributed in 17 farms located in São Paulo, as described in figure 4.

Environmental data collected by using the Thermo-Hygro-Anemometer in each farm visit are shown at Table 2. The comparison between the environmental data according to the season did not detected difference for wind speed, temperature and heat index parameters, but humidity presented difference in the middle day ($p=0.03$) and afternoon ($p<0.01$) between spring/summer and winter season, with higher humidity levels at middle day and afternoon at the hottest season. In the morning, humidity was similar between seasons. Dew point were statically higher in middle day ($p=0.01$) and afternoon ($p<0.01$) at summer/spring.

Table 2 – Environmental data assessed at pen calves, wind speed (m/s), temperature (ºC), chill (ºC), humidity (%), heat index and dew point (ºC) on the farms at the visit day

	WINTER			SUMMER/SPRING			*P*-VALUE (BETWEEN SEASON)
PARAMETERS	**Mean**	**Median**	**Range**	**Mean**	**Median**	**Range**	
V Max (m/s)							
Morning	0.23	0.0	0-0.9	0.60	0.5	0.4-0.9	0.14[2]
Middle Day	1.20	0.9	0.5-3	0.84	1	0-1.4	0.79[2]
Afternoon	1.03	0.85	0-3.6	0.51	0.3	0-1.5	0.20[2]
V Mean (m/s)							
Morning	0.07	0	0-0.4	0.27	0.3	0-0.5	0.09[2]
Middle Day	0.56	0.45	0.3-1.2	0.52	0.5	0-1.1	0.96[2]
Afternoon	0.56	0.5	0-2.9	0.31	0.5	0-0.7	0.69[2]
T (ºC)							
Morning	19.54	18.2	12.9-26.7	24.87	25.8	21.1-27.7	0.09[1]
Middle Day	27.85	27.2	20.9-31.9	29.12	29.7	23.7-32.7	0.51
Afternoon	29.74	29.05	22.5-39.3	27.83	27.3	24.1-32	0.24
Chill (ºC)							
Morning	19.40	18.05	12.7-26.6	24.83	25.8	21-27.7	0.09[1]
Middle Day	27.72	27.3	20.7-31.9	29.02	29.7	23.8-32.1	0.49[1]
Afternoon	29.66	29.05	22.4-39.3	27.71	27	24.1-31.9	0.23[1]
Humidity (%)							
Morning	69.02	70.45	48.2-86.5	71.20	71.4	63.5-78.7	0.70[1]
Middle Day	49.92	50.95	31.8-67.3	70.96	75.3	49.9-86.5	0.03[1]
Afternoon	36.93	34.4	25.5-63	67.13	68.1	49.1-79.5	<0.01[2]
HI							
Morning	19.26	17.8	12.6-26.5	26.27	28.1	21.1-29.6	0.10[1]
Middle Day	28.20	28.55	20.6-32.8	32.66	33.5	25.3-37.3	0.10[1]
Afternoon	29.03	28.2	22.4-36.8	30.20	29.8	24.6-34.9	0.54[1]
DP (ºc)							
Morning	13.58	14.1	9.4-18	19.20	20.3	15.3-22	0.09[1]
Middle Day	15.51	15.55	13.1-18.2	21.48	21.2	19.5-23.9	0.01[1]
Afternoon	12.49	13.25	8.4-15.6	20.96	21.1	18-22.9	<0.01[1]

V max (m/s): maximum wind speed; V mean (m/s): mean wind speed; T: Temperature; HI: Heat Index; DP: Dew Point.
Morning: 7am to 10am; Middle Day: 11 am to 1 pm; Afternoon: 2pm to 7pm
[1]Paired t-test at parametric data
[2]Mann-Whitney for non parametric data
Source: DECARIS, 2021

The distribution of the prevalence (%) of BRD, detected by the different diagnosis methods performed in this experiment are shown on Table 3. The highest prevalence of BRD between farms presented variations according to the diagnosis methods performed. The highest prevalence of respiratory disease considering the auscultation findings and lung consolidation was detected in farm 9. On the other hand, the high prevalence of BRD based on Wisconsin and California score were observed in the farm 14 (85%) and farm 8 (51%), respectively. Finally, the prevalence of calves positive for haptoglobin (≥15 mg/dL) was 100% at the farms 2, 5, 7, 9 and 13. The maximum median depth of consolidation observed in this study was 4.99 cm in an animal from farm 17. Supplementary figures 1, 2 and 3 presented TUS images obtained at the study.

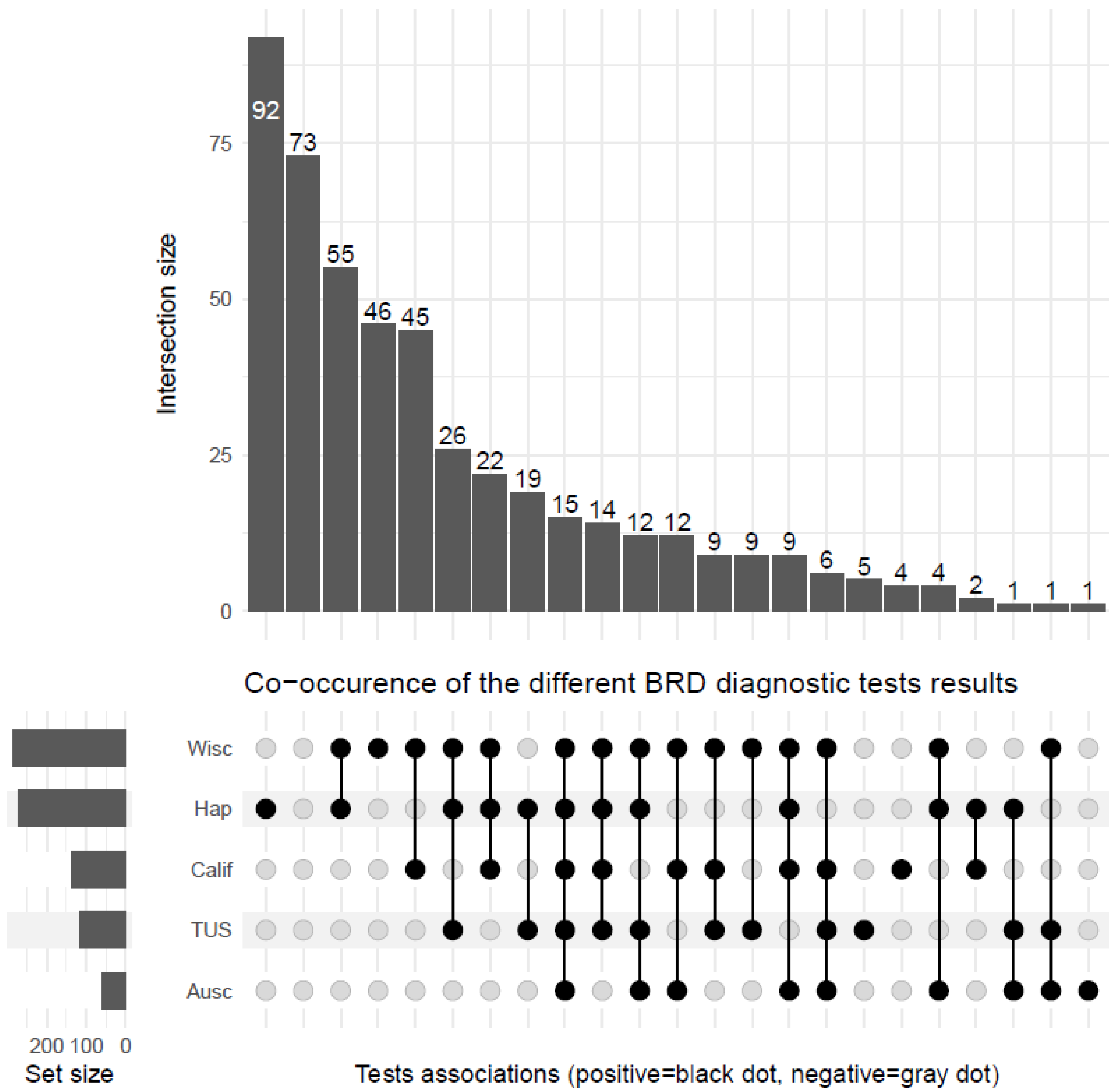
Table 3 - Distribution of age, positivity at the five methods to detect BRD (TUS, Wisconsin and Californian BRD Scores, Haptoglobin), median of consolidation depth according to the results in lungs ultrasound examination, by each farm

FARM	N[1]	AGE (DAYS)[2]	AUSC+ (%)[3]	TUS + (%)[4]	DEPTH CONS (cm)[5]	WISC+ (%)[6]	CALIF+ (%)[7]	HAP+ (%)[8]	HAP RANGE (mg/dl) [9]
1	15	63 ±16,2	0%	20%	1.30 - 4.22	40%	20%	20%	1.06 - 40.52
2	15	71 ±23,4	13%	20%	1.17 - 3.09	60%	27%	100%	16.21 - 38.6
3	38	78 ±23,5	3%	8%	1.00 - 3.11	42%	8%	53%	2.22 - 81.02
4	36	51,5 ±13,3	11%	19%	1.31 - 4.40	50%	33%	33%	1.72 - 93.41
5	28	63,5 ±15,9	21%	46%	1.00 - 4.48	68%	25%	100%	27.07 - 76.41
6	12	43 ±10,1	8%	8%	4.41 - 4.41	42%	17%	17%	1.03 - 20.67
7	9	46 ±13,3	11%	0%	-	44%	11%	100%	15.59 - 20.82
8	37	42 ±7,9	14%	35%	1.02 - 4.58	81%	51%	0%	1.29 - 8.14
9	50	52 ±14,6	32%	72%	1.00 - 4.71	82%	30%	100%	26.3 - 145.82
10	10	46,5 ±10,8	0%	10%	3.66 - 3.66	60%	40%	0%	0.92 - 4.77
11	19	49 ±9,7	11%	5%	1.30	58%	21%	32%	5.01 - 21.89
12	17	72 ±19,6	6%	0%	-	65%	18%	88%	4 - 90.91
13	5	60 ±6,8	0%	40%	1.37 - 3.61	80%	40%	100%	16.1 - 19.17
14	13	71 ±18,4	15%	54%	1.14 - 3.86	85%	23%	92%	8.83 - 43.54
15	89	48 ±11	9%	17%	1.27 - 4.07	69%	37%	52%	1.18 - 73.89
16	69	60 ±23,3	13%	10%	1.07 - 4.86	55%	20%	68%	0.49 - 218.32
17	20	49 ±12,3	25%	25%	1.25 - 4.99	75%	45%	10%	1.31 - 21.06

[1] Number of enrolled calves
[2] Median ± SD
[3] Ausc+: Presence of crackles and wheezes
[4] TUS+: Positivity at thoracic ultrasound. Consolidation was defined based as the presence of at least 1 site with lung consolidation depth ≥1 cm.
[5] Range of consolidation depth measured at ultrasonographic scanning
[6] Wisc+: Positivity at Wisconsin BRD score, when clinical signs sum ≥4
[7] Calif+: Positivity at Californian BRD score, when clinical signs sum ≥5
[8] Hap+: Positivity with serum concentrations ≥15mg/dL
[9] Range of measured Hap concentration in each farm.
Source: DECARIS, 2021

The distribution of calves classified as a positive according to the diagnosis methods are shown in the Supplementary Table 1 and Figure 6. It was found 56.2% (271/482), 24.3% (117/482), 59.1% (285/482), 28.6% (138/28.6%) and 12.6% (61/482) of positive heifers for haptoglobin, TUS, Wisc, Calif and Ausc, respectively. According to the combination of methods, the most of animals enrolled in this experiment had positive results only for haptoglobin test or combination between haptoglobin and Wisconsin Score System.

Figure 6 – Repartition of 482 preweaned dairy calves based on the 32 bovine respiratory disease (BRD) tests (Ausc, TUS, Wisconsin and Californian BRD Scores, Haptoglobin) combinations results.

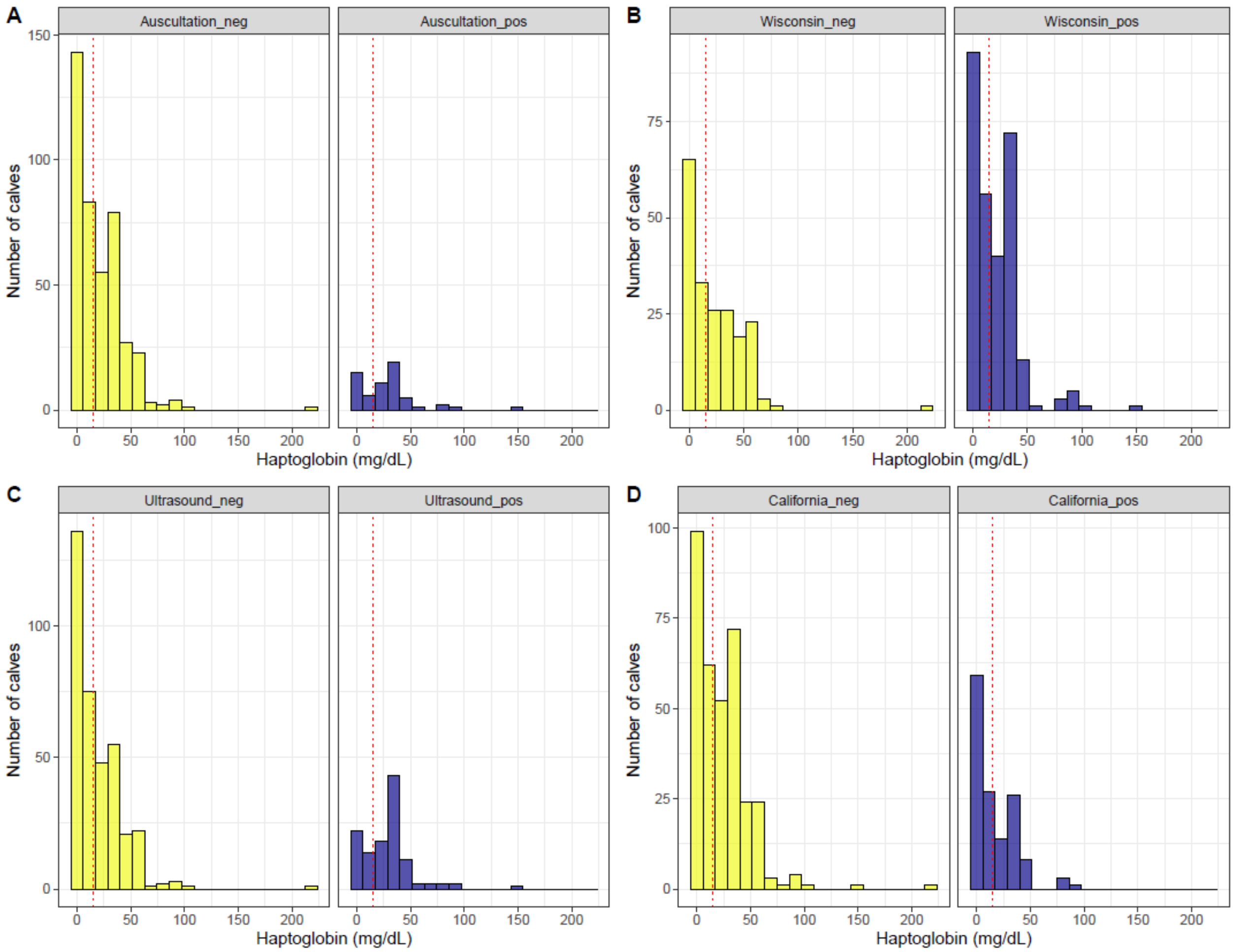


Source: DECARIS, 2021

The histograms presented in the figure 7 shows the distribution of positive and negative calves for TUS, BRD Scoring Systems and Ausc, according to the haptoglobin concentration. The most of calves negative for auscultation, TUS, Wisconsin and

California score had haptoglobin values above the cut of ≥ 15 mg/mL as well positive animals for the different diagnosis BRD methods.

Figure 7 - Histogram distribution of 482 preweaned dairy calves according to haptoglobin concentration for positive and negative outputs of the different tests (Ausc, TUS, Wisconsin and Californian BRD Scores) results.



Red line represents the threshold to consider Hap positive (>15mg/dL)
Source: DECARIS, 2021

The posterior findings of the main BLCMand the model with non-informative prior performed in this study are summarized in Table 4. The 95% Bayesian credible intervals of the different tests sensitivities and specificities are depicted in figure 5. The Wisconsin BRD Scoring System has a high Se, but low Sp median estimate of 0.97 (95% BCI: 0.89-0.99) and 0.52 (95% BCI: 0.46-0.59), respectively. California BRD Scoring System presented more balanced estimates for Se and Sp of 0.6 (95% BCI: 0.47-0.72) and 0.81 (95% BCI: 0.76-0.85), respectively (Figure 8).

Table 4 – Posterior median and 95% credible intervals based on 2 Bayesian latent class models to determine the accuracy of 5 different diagnostic tests to diagnose BRD in Holstein female calves.

	POSTERIORS MODEL 1			POSTERIORS MODEL 2		
PARAMETERS	**Prior**	**Median**	**95% BCI**	**Prior**	**Median**	**95% BCI**
SE-AUSC	beta(0.69,3.55)	0.507	0.375-0.672	beta(1,1)	0.563	0.384-0.834
SP-AUSC	beta(4.9,0.84)	0.992	0.969-1	beta(1,1)	0.992	0.972-1
SE-TUS	beta(23.9, 5.04)	0.677	0.572-0.778	beta(1,1)	0.600	0.468-0.725
SP-TUS	beta(42.57, 5.62)	0.878	0.832-0.921	beta(1,1)	0.855	0.801-0.906
SE-HAP	beta(21.0, 11.8)	0.680	0.587-0.767	beta(1,1)	0.666	0.544-0.778
SP-HAP	beta(47.5, 20.9)	0.510	0.461-0.561	beta(1,1)	0.466	0.412-0.522
SE-WISC	beta(1,1)	0.973	0.894-0.999	beta(1,1)	0.977	0.914-0.999
SP-WISC	beta(1,1)	0.525	0.466-0.588	beta(1,1)	0.517	0.452-0.594
SE-CALIF	beta(1,1)	0.600	0.476-0.724	beta(1,1)	0.638	0.507-0.765
SP-CALIF	beta(1,1)	0.807	0.758-0.855	beta(1,1)	0.809	0.756-0.869
Π	beta(1,1)	0.232	0.168-0.308	beta(1,1)	0.216	0.138-0.312
COVDN	U(0,a)	0.083	0.061-0.105	U(0,a)	0.081	0.054-0.103
COVDP	U(0,b)	0.009	-0.004-0.049 U(0,b)	U(0,b)	0.007	-0.004-0.041
DIC		157.1			154.5	

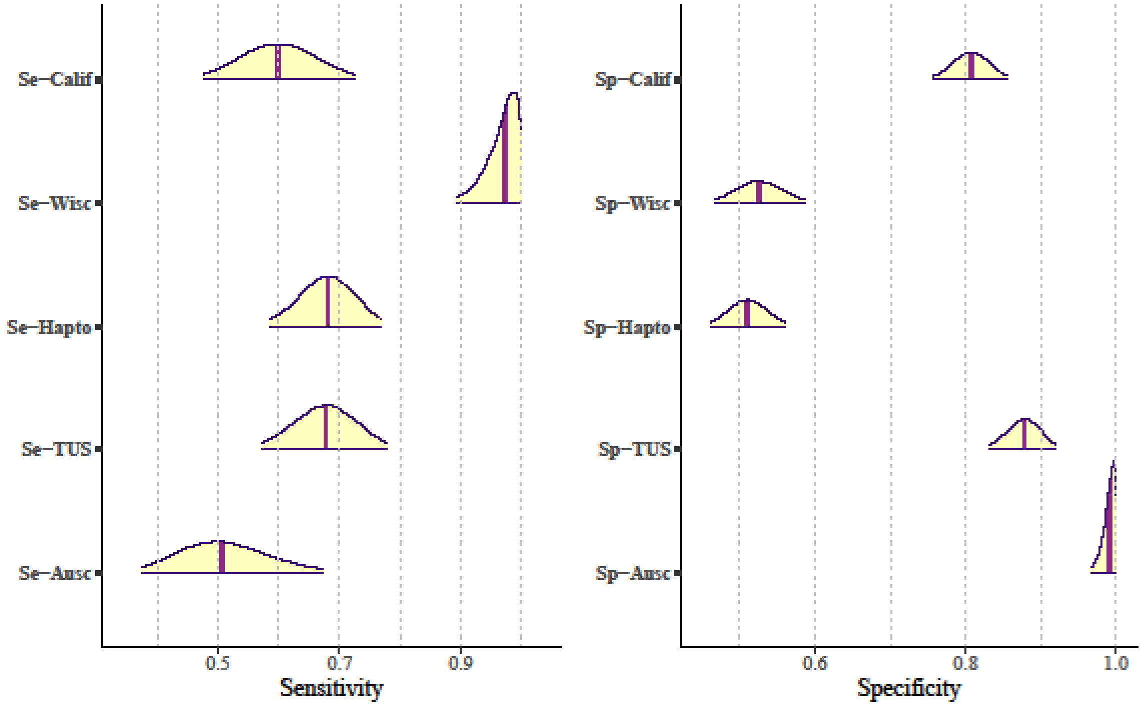
The sensitivities (Se) and specificities (Sp) of five different tests (thoracic auscultation (Aus), thoracic ultrasound (TUS), Haptoglobin (Hap), Wisconsin score (Wisc) and Californian score (Calif). The informative priors used for model 1 are based on previous published literature. The model 2 were non informative prior (uniform probability between 0 and 1 (beta(1,1) or U(0,1))

covDp, covDn : covariance of the Wisconsin and Californian score accuracy positive (p) or negative (n) BRD cases.

a=min(Se-Wisc, Se-Calif)- (Se-Wisc* Se-Calif), b= min(Sp-Wisc, Sp-Calif)- (Sp-Wisc* Sp-Calif)

Source: DECARIS, 2021

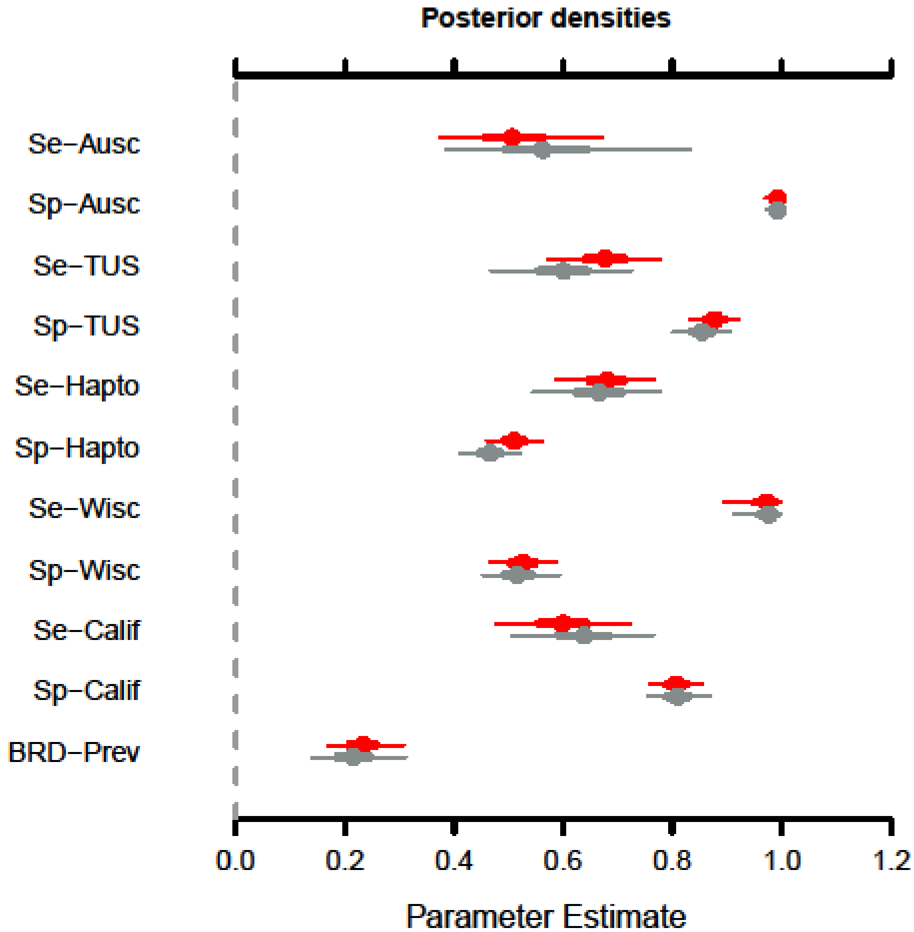
Figure 8 – Density intervals of sensitivity and specificity of the five different tests (Ausc, TUS, Wisconsin and Californian BRD Scores) to diagnose BRD in Holstein female calves.



Source: DECARIS, 2021

The sensitivity analysis did not reveal clinically important difference between the main and non-informative model (Figure 9).

Figure 9 - Intervals of sensitivity and specificity for each five different tests (Ausc, TUS, Wisconsin and Californian BRD Scores) to detect BRD in the two Bayesian latent class models.



Red lines represent Model 1, grey line represent Model 2. The dot represent the median estimate, the thick line represents the 50% Bayesian credible (BCI) and the thin line represents the 95% BCI.
Source: DECARIS, 2021

5 DISCUSSION

This research evaluated the accuracy of thoracic auscultation, thoracic ultrasound and two available scores to diagnosis BRD on preweaned calves in subtropical conditions.

Data of our previous studies indicated a high frequency of dairy calves positive for respiratory disease applying the Wisconsin BRD Scoring System, and it was possible to observe that the rectal temperature was the main parameter responsible for the increase of the BRD score sum (≥ 5.0) (GOMES, 2021). Based on our previous results, we formulate the hypothesis that the environmental condition and weather from Wisconsin compared with tropical and subtropical countries could invalidate the use of Wisconsin and/or California BRD Scoring System. Köppen-Geiger climate classification São Paulo is Cfa characterized by warm temperate, fully umid with hot summer; California is classified as Csc, characterized by warm temperate, dry summer, cool summer and cold winter; and Wisconsin is classified as Dfa, characterized by snow zone, fully humid and warm summer (KOTTEK et al., 2006).

The environmental data from our research was characterized by a cold and hot season, observing higher humidity and dew point at spring and summer than winter. On the other hand, there were any differences for wind speed, temperature and heat index between seasons. As expected São Paulo state presented higher temperatures over the year, differently at Wisconsin state as described by Lago et al. (2006), characterized by low temperatures in the winter (3.9°C±6.7 to 12.2°C) and high wind speed (2,68 m/s). Harper et al. (2009) described the weather in Wisconsin state as a cold winter with temperatures range from −20 up to 5°C, and mild summer with temperatures from 18 up to 23°C. Californian temperatures at summer vary from 7.7ºC to 41.9ºC associated with low humidity (LOUIE et al., 2018).

São Paulo state weather profile marked by high temperatures and humidity as presented in part of Texas, Australia, Portugal, Italy, Japan and Argentina. Thermoneutral zone for calves range from 0ºC up to 26ºC, and animals raised in subtropical and tropical countries could exhibit an increased respiratory rate as a thermoregulatory mechanisms to dissipate heat (ROLAND et al., 2016). Heat stress in calves was observed in an ambient temperature above 32°C and a relative humidity above 60% (Neuwirth et al. 1979). The range of temperature observed in our experiment was 22.5 up to 39.3 in the winter season and 24.1 up to 32.0°C, so the exanimated dairy calves could have a compensatory tachypnea. Tt is known that rectal temperature increase in animals exposed to high environmental temperature, and decrease with high relative humidity and wind velocity

(ROLAND et al., 2016). Also, based on these considerations middle day and afternoon times are worsts moments to assess calves for scoring system as environmental temperatures influence the rectal temperature and respiratory rate. So, data from rectal temperature and tachypnea should be interpretate and used with caution in the diagnosis dairy calves as positive for BRD.

The accurate diagnosis of bronchopneumonia, discerning between upper and lower airway disease, is a challenge due to the lack of specific clinical signs. Despite of that, it is important to establish a treatment plan timely to avoid the negative effects of the disease and reduce the unjustified and inappropriate use of antimicrobials. In this scenario timely and precisely detection of disease is a key point to manage BRD in dairy calf operations. Another big challenge of diagnosing BRD is the absence of a gold standard.

The cut-off value used to classify calves as a positive for haptoglobin in our study was 15.0mg/dL, based on the results previously presented by Svensson et al. (2007). These authors used a commercial kit for the quantification of haptoglobin, and we have used an in house protocol. Despite of that, both of these techniques are turbidometric assay based on the haptoglobin ability to bind with hemoglobin.

The major number of calves from this study had positive results only for haptoglobin (concentration ≥ 15 mg/dL), and this population of calves was higher than the 32 other combinations. Haptoglobin is the major acute phase protein in bovine, as others acute phase protein is sensitive and non-specific biomarker used to detect infection and inflammation (ECKERSALL; BELL, 2010). The concentration of haptoglobin is increased in calves manifesting BRD, compared with healthy animals (JOSHI et al., 2018; MOISÁ et al., 2019). The non-specific profile of the increase of haptoglobin could be justify the high percentage of positive dairy calves observed in our study. The target age investigated in our study is susceptible for umbilical inflammation, diarrhea, babesia, anaplasmosis and keratoconjunctivitis (WINDEYER et al., 2014; GOMES, 2021), and these bunch of disease could be a cause of the high proportion of positive animals (haptoglobin ≥ 15 mg/dL) in our study. Haptoglobin is not capable to differentiate these sickness conditions, however, it is important to highlight that haptoglobin level can distinguish health and sick calves (MURRAY et al., 2014). An interesting observation in this study was the wide variation for haptoglobin levels according to the investigate farms, indication that haptoglobin has potential to be a herd-level indicator.

The non-specific profile of haptoglobin could justify the low Se and Sp, 66.6% and 46.6%, respectively, detected in our study. Svenson et al. (2007) investigated different cut-off values to detect BRD diagnosed based on physical examination and pulmonary auscultation, and these authors found Se and Sp equal 0.64 and 0.71, respectively. Idoate et al*.* (2015) have evaluated the effectiveness of haptoglobin to detect respiratory disease in feedlot cattle by using the Wisconsin respiratory score, and they indicated the cut-off value ≥0.81 mg/mL defined by ROC curve. (Se 92.86% and Sp 85.71%). Previous study in California dairies (n=477 calves), 194 BRD cases confirmed by TUS or Ausc, and 283 calves were control group, Hap obtained a Se 46.4% and Sp 81.6%, the cut-off of Hap ≥19.5 mg/dL was determined by ROC Curve. (MOISÁ et al., 2019). The literature available regard the use of acute phase proteins as a diagnostic method to detect BRD is scarce, observing high variation between previously results, due to the different designs and BRD diagnosis methods employed in this different experiment.

Normal breath sounds in lungs area are formed by the air turbulence in airways ramifications, and it is affected by velocity of airflow and diameter of airway, these sounds are clearly audible over the trachea and attenuated over the lungs. Increase loudness of breath sounds, caused by increase respiratory rate and depth of respiration can occur at physiological reasons (exercise, high environmental temperature) or at early pneumonia and consolidation. Pathological respiratory sounds detected in BRD cases could be silence areas, caused consolidated areas without airflow, crackles, caused by intense deviation of airflow by obstruction by secretion and exudate, and wheezes, formed by airflow acceleration caused by increased pulmonary density or airway stricture (STÖBER; DIRKSEN; GRÜNDER, 1992; CONSTABLE et al., 2017b). In our study, the abnormal sound considered in the bronchopneumonia classification was crackles, wheezes and silence areas.

In our study, thoracic auscultation method presented Se and Sp equal 0.563 and 0.992, respectively. Bucczinki et al. (2014a) used as criteria for abnormal lung sounds crackles, wheezes, and pleural friction rubs and the absence of respiratory noises, and observed sensitivity was 5.9% (0.0% to 16.7%), and specificity was 98.8% (97.3% to

100.0%), lower Se and equal Sp comparing to our results. These differences might be caused by the previous study concentrate the examination at middle and ventral parts of the thorax, focusing at the most common locations of bronchopneumonia, as we examinate all calve lung. Buczinski et al. (2016) used a Bayesian framework to estimate accuracy of auscultation and found high sensitivity, 72.9%, and lower specificity, 53.3%, compared with the results from our research, using as Ausc+ criteria the presence of increased bronchial sounds or presence of abnormal sounds as crackles and wheezes or total absence of lung sounds. The criteria used to these research and ours, might cause the observed difference, because excluding the increase on the bronchial sounds as abnormality, we can reduce physiological reasons that can cause this phenomenon as anxiety, fever, exercise, tachypnea and high environmental temperature as a factor to cause positive results in the auscultation, reducing the test specificity (CONSTABLE et al., 2017b). But in other hand, our lack of sensitivity compared to the previous research could be caused by excluding the abnormalities, because it is the commonly the first abnormal lung sound to appear in animals with BRD (BUCZINSKI; MÉNARD; TIMSIT, 2016). Veterinarians commonly use thoracic auscultation to confirm bronchopneumonia, but even an experiment operator, the sensitivity of this test to detect lung disease seems poor, and adding increased bronchial sounds in the positivity of auscultation improve the Se while reduce Sp.

Thoracic ultrasound earned highlights in the detection of BRD, because it is a practical method, portable, easily to perform in farm, with a good inter-operator agreement to detect lung consolidations (BUCZINSKI et al., 2018a). In this study, the Se and Sp for the TUS was 60.0% and 85.5%, respectively, considering the consolidation lesions with ≥ 1 cm of depth as positive. Estimated accuracy of TUS in Bayesian approaches in literature was Se 79.4% and Sp 93.9% (BUCZINSKI; L OLLIVETT; DENDUKURI, 2015), Se 76.5% and Sp 92.9% (BUCZINSKI; MÉNARD; TIMSIT, 2016), when considering lesion with ≥ 1 cm of depth as positive. Estimated accuracy of TUS was obtained by Berman et al, (2020) using criteria for positive calves as the presence of lung consolidations, pneumothorax, pleural effusion, or cavitary lesion and estimated Se and Sp was 81% and 90%, respectively. All previous approaches presented higher Se and Sp accuracy than our results. This could lead because lung consolidation is not the unique ultrasonographic finding in cases of respiratory disease. B-lines, pleural effusion, pneumothorax are also found in cases of pneumonia, and the lung consolidation can be absent at pneumonia caused by viral pathogens and early stages of the disease (BUCZINSKI; L OLLIVETT; DENDUKURI, 2015). The criteria of only consolidation ≥1 cm of depth to characterize the bronchopneumonia could cause the loss of Se. Also, Berman et al, (2019) reported that adding the right cranial site increased TUS Se, but with an lack of Sp, to detect active pneumonia this could be a reason that our presented specificity was lower than previous studies, since our evaluation was since the 1st intercostal space.

BRD scores systems are an important tool to screening sick animals, an easy management practice to implement in a farm, but the systems fail in differentiate upper and lower respiratory tract disease and does not identify subclinical cases (OLLIVETT; BUCZINSKI, 2016). At the scores level the Wisc Score presented high positive outcomes, all farms presented more than 40% of evaluated calves positive for BRD, and total positivity for BRD at Wisc Score was 59.2% (285/482), our results waslower in genral at recent study of our research group presented 73% (438/600) of calves positive to BRD using Wisc score as diagnostic tool (GOMES, 2021), but some farms at our research (8,9,13,14,17) presented higher outcomes than reported.

The estimated accuracy of Wisconsin score Se was 97.7% (91.4 - 99.9), however this scoring system presented very low Sp equal 51.7% (45.2-59.4). Previously study developed in Canada reported different values for Se 62.4% and Sp 74.1% using the same scoring system and Bayesian statistical methods to investigate the accuracy of different BRD tests (BUCZINSKI; L OLLIVETT; DENDUKURI, 2015). Californian score was developed because of lack previous scores using quantitative methods to attribute the weights to clinical signs at score system, and to be a pratical and simple score to use at farm level (LOVE et al., 2014). In our study, Californian score presented Se 63.8% (50.7-76.5) and Sp 80.9% (75.6-86.9), showing more balance accuracy compared with the Wisconsin score.

Since we know this is the first research assessing the Bayesian accuracy of Californian score. Agreement between the both scores was performed by Aly et al (2014), and the kappa coefficient obtained was 0.85, what was expected, since the development of Calif score used Wisc score as part of the classification of positive and negative calves.

Love et al, (2016) assessed the accuracy of Wisc and Californian scores using ultrasound and auscultation to detect positive calves and observed Wisc Screening Se 46,0%, Diagnostic Se 72,6% and Sp 91,2%, Calif Screening Se 46,0%, Diagnostic Se 71,1% and Sp 87,4%, but at this study calves were select as clinical apparent cases and randomly selected cases, this may lead at differences in Se because of the severity of clinical signs. Buczinski et al (2018b) used Bayesian model to update the points for each parameter resulting in different weights as proposed in the development of Calif score, the points were distributed in 20 points for dyspnea, 7 points temperature ≥39.2ºC, -1 point to eye discharge, 10 points to nasal discharge, 16 points to ear drop or head tilt and 16 points to spontaneous cough, and the optimal cutoff in the different scenarios were 9 to 16 with Se varying from 83.0 to 66.9% and Sp from 69.1 to 82.7% respectively.

This profile shows that Wisc score was more affected by the environmental differences comparing where the score was created and Brazil. Californian score presented more balance results for Se and Sp, with dichotomous cut-point for the variables established as positive when clinical signs were more significant. Important point to observe is that the operator for Californian score need to differentiate the tachypnea, common in heat stress, and dyspnea, when not only respiratory movements become more prominent, it could include the extension of the head and neck, dilatation of the nostrils, abduction of the elbows, and breathing through the mouth adding increased movement of the thoracic and abdominal walls (CONSTABLE et al., 2017a), to not super estimate the number of animals with abnormal respiration that could cause a higher score punctuation.

A lack of this study was not visiting the same farms at winter and summer seasons, this could be an interesting observation of environmental exposure for the calves in the both season in each farm, to observe and compare the incidence of BRD, and evaluate the performance of the tests. Diagnosis of disease that could affect animal at this age range such as diarrhea, anaplasmosis and babesiosis complex, keratoconjutivitis, could affect the evaluation of scores. Another point that could be explored at future studies is the cut point of Haptoglobin to detect BRD including the presence of concomitant diseases that could increase the acute phase protein.

6 CONCLUSION

The current study estimated the Se and Sp of five test to diagnose BRD in calves. Hap presented moderate Se and low Sp, resulting in a difficult test to use in our condition, Ausc presented high Sp but low Se even when performed by experienced professional. Previous developed scores (California and Wiscosin), presented inverse profiles the adaptation for a new threshold or a creation of a new score system for countries with high humidity and temperature could reduce the lack of sensitivity and specificity in these specific conditions for these developed methods. TUS presented moderate Se and higher Sp, and is an easier method to perform at farm level, they might be associated after a high Se screening method to confirm the presence of the disease.

Our results shows that there is no test available sufficiently specific or sensitive to diagnosis calves bronchopneumonia alone, interpretate tests in parallel should reduce the number of false positive and false negative animals.

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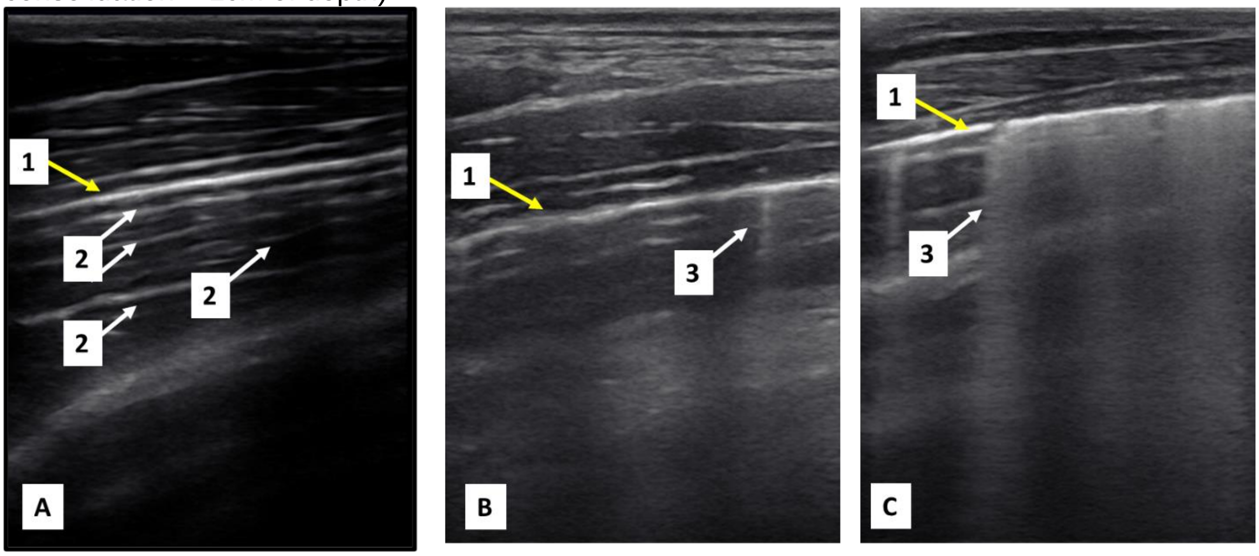
SUPPLEMENTARY TABLES & FIGURES

Supplementary Table 1 – Distribution of the calves in the 32 possible combinations according to the positive (Pos) or negative (Neg) results in the different diagnosis methods used to detected bronchopneumonia (Ausc, TUS, Wisconsin and Californian BRD Scores, Haptoglobin)

Possibilites	Ausc[1]	TUS[2]	Hap[3]	Wisc[4]	Calif[5]	N[6]
1	Neg	Neg	Neg	Neg	Neg	73
2	Neg	Neg	Neg	Neg	Pos	4
3	Neg	Neg	Neg	Pos	Neg	46
4	Neg	Neg	Neg	Pos	Pos	45
5	Neg	Neg	Pos	Neg	Neg	92
6	Neg	Neg	Pos	Neg	Pos	2
7	Neg	Neg	Pos	Pos	Neg	55
8	Neg	Neg	Pos	Pos	Pos	22
9	Neg	Pos	Neg	Neg	Neg	5
10	Neg	Pos	Neg	Neg	Pos	0
11	Neg	Pos	Neg	Pos	Neg	9
12	Neg	Pos	Neg	Pos	Pos	9
13	Neg	Pos	Pos	Neg	Neg	19
14	Neg	Pos	Pos	Neg	Pos	0
15	Neg	Pos	Pos	Pos	Neg	26
16	Neg	Pos	Pos	Pos	Pos	14
17	Pos	Neg	Neg	Neg	Neg	1
18	Pos	Neg	Neg	Neg	Pos	0
19	Pos	Neg	Neg	Pos	Neg	0
20	Pos	Neg	Neg	Pos	Pos	12
21	Pos	Neg	Pos	Neg	Neg	0
22	Pos	Neg	Pos	Neg	Pos	0
23	Pos	Neg	Pos	Pos	Neg	4
24	Pos	Neg	Pos	Pos	Pos	9
25	Pos	Pos	Neg	Neg	Neg	0
26	Pos	Pos	Neg	Neg	Pos	0
27	Pos	Pos	Neg	Pos	Neg	1
28	Pos	Pos	Neg	Pos	Pos	6
29	Pos	Pos	Pos	Neg	Neg	1
30	Pos	Pos	Pos	Neg	Pos	0
31	Pos	Pos	Pos	Pos	Neg	12
32	Pos	Pos	Pos	Pos	Pos	15

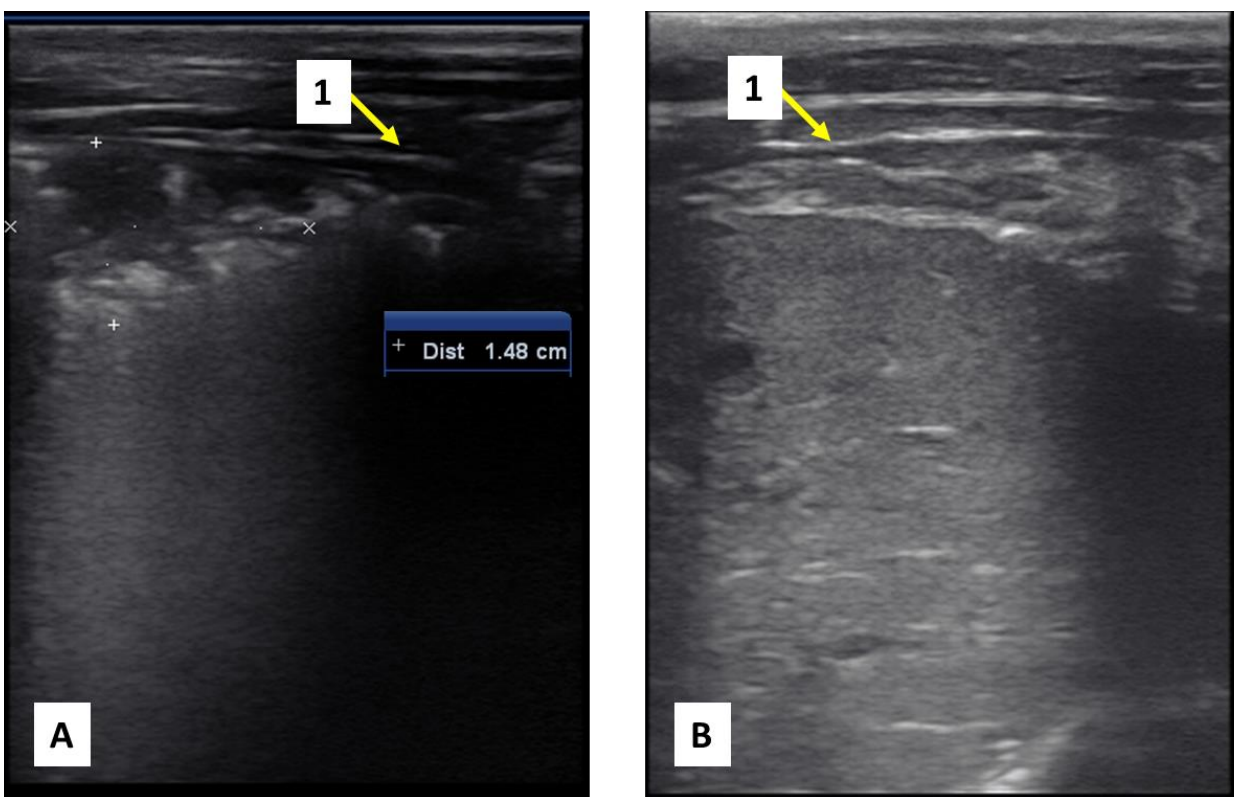
[1] Ausc: Positivity defined as presence of crackles, wheezes, rhonchi.
[2] TUS: Consolidation (positivity at TUS) was defined based as the presence of at least 1 site with lung consolidation depth ≥1 cm.
[3] Hap: Defined positive when serum concentration was ≥15mg/dL
[4] Wisc: Positivity when sum ≥4
[5] Calif+: Positivity when sum ≥5
[6] Number of animals

Supplementary Figure 1 – Thoracic ultrasound images classified as normal (no consolidation, or consolidation < 1cm of depth)



A – Normal lung image; 1- Pleura; 2 – A-lines (reverberation artefacts)
B – Presence of discrete B-line; 1 – Pleura; 3 – B-line or Comet tail artefacts (CTA)
C – Diffuse CTA artefacts imaging, with no sign of consolidation; 1 – Pleura; 3 - B-line or Comet tail artefacts (CTA)

Supplementary Figure 2 – Thoracic ultrasound images classified as abnormal (consolidation ≥ 1cm of depth)



A – Consolidation with 1.48 cm of depth; 1 – Pleura
B – Consolidation of cranial lobe; 1 – Pleura

Supplementary Figure 3 – QR Codes of normal lung TUS video (A), and consolidated lung TUS video (B)

(A) TUS - Normal Lung

(B) TUS – Consolidated Lung