

Associations between clinical manifestations and respiratory pathogen detections observed in vaccinated cattle from Brazil¹

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ABSTRACT.- Headley SA, Fenta MD, Fritzen JT, Madeira RR, Machado BM, Oliveira O, Marques MS, Yoshitani GD, Silva FHP, Deritti GO, Reis MH, Cunha Filho LFC, Baptista AL, Alfieri AA. **Associations between clinical manifestations and respiratory pathogen detections observed in vaccinated cattle from Brazil.** *Pesquisa Veterinária Brasileira* 46:e07882, 2026. Laboratory of Animal Pathology, Department of Preventive Veterinary Medicine, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, PR-445 Km 380, Campus Universitário, PR 86057-970, Brazil. E-mail: selwyn.headley@uel.br

Bovine respiratory disease (BRD) remains a significant challenge in feedlot systems and involves the complex interaction of viral and bacterial pathogens. There are few studies from Brazil that have investigated possible associations between pathogen detection and clinical manifestations of BRD. This cross-sectional study investigated pathogen detection and the molecular evidence of possible post-vaccination detection of vaccine-included pathogens (PVD-VIP) in feedlot cattle immunized at entry with an inactivated respiratory vaccine [*Histophilus somni*, *Pasteurella multocida*, *Mannheimia haemolytica*, bovine alphaherpesvirus 1 (BoAHV1), bovine parainfluenza virus 3, bovine viral diarrhea virus] at three feedlots from distinct geographical regions of Brazil. Deep nasopharyngeal swabs (DNS) were randomly collected from 37 steers for the molecular detection of 10 BRD pathogens (including bovine coronavirus [BCoV], ovine gammaherpesvirus 2, bovine respiratory syncytial virus, and *Mycoplasma bovis* that were not contained in the vaccine). In the absence of a non-vaccinated group, detections of vaccine-included pathogens were interpreted as potential PVD-VIP and evidence of pathogen circulation. Clinical signs were assessed by the modified DART system and manifestations of disease in cattle. Multivariable analysis and multinomial logistic regressions evaluated possible clinical-pathogen detection patterns. Single-pathogen detection was predominant (24.3%; 9/37) over dual (10.8%; 4/37) and triple (2.7%; 1/37) detections, without significant differences among feedlots. BCoV was the most frequently detected (18.9%;

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7/37), occurring in singular and mixed detections (including the only triple detection: BCoV + *M. haemolytica* + *H. somni*). None of the 10 pathogens investigated were detected in 62.2% (23/37) of DNS. Potential PVD-VIP agents identified were *P. multocida* (8.1%; 3/37), BoAHV1, *H. somni*, and *M. haemolytica* (each 5.4%; 2/37). Exploratory analyses identified potential associations between profuse salivation and *P. multocida*, stunted growth and BoAHV1, and fever and BCoV. However, due to the reduced sample size, these associations require confirmation with larger cohorts. Nonetheless, these results demonstrated that the vaccinated animals at these feedlots developed potential PVD-VIP and suggest that PVD-VIP with BRD-compatible clinical presentations in vaccinated cattle may occur despite immunization against BRD agents.

INDEX TERMS: Bovine alphaherpesvirus 1; bovine coronavirus; bovine respiratory disease; detection patterns; *Histophilus somni*; infection modelling; *Mannheimia haemolytica*; molecular detection.

RESUMO.- [Associações entre manifestações clínicas e detecções de patógenos respiratórios observadas em bovinos de vacinados no Brasil] A doença respiratória bovina (DRB) continua sendo um desafio significativo em sistemas de confinamento e envolve a interação complexa de patógenos virais e bacterianos. Há poucos estudos no Brasil que investigaram possíveis associações entre a detecção de patógenos e as manifestações clínicas da DRB. Este estudo transversal investigou a detecção de patógenos e a evidência molecular de possíveis detecção pós-vacinação de patógenos incluídos na vacina (DPV-PIV) em bovinos de confinamento imunizados na entrada com uma vacina respiratória inativada [*Histophilus somni*, *Pasteurella multocida*, *Mannheimia haemolytica*, alfa herpesvírus bovino 1 (BoAHV1), vírus parainfluenza bovino 3 e vírus da diarreia viral bovina] em três confinamentos de distintas regiões geográficas do Brasil. Swabs nasofaríngeos profundos (SNP) foram coletados aleatoriamente de 37 novilhos para a detecção molecular de 10 patógenos associados à DRB (incluindo coronavírus bovino [BCoV], gama herpesvírus ovino 2, vírus sincicial respiratório bovino e *Mycoplasma bovis*, que não estavam contidos na vacina). Na ausência de um grupo não vacinado, as detecções de patógenos incluídos na vacina foram interpretadas como potenciais detecção pós-vacinação de patógenos incluídos na vacina e evidência de circulação de patógenos. Os sinais clínicos foram avaliados pelo sistema DART modificado e pelas manifestações de doença em bovinos. Análises multivariáveis e regressões logísticas multinominais avaliaram possíveis padrões clínico-diagnósticos de detecção de patógenos. A detecção de patógeno único predominou (24,3%; 9/37) em relação às detecções duplas (10,8%; 4/37) e triplas (2,7%; 1/37), sem diferenças significativas entre os confinamentos. O BCoV foi o patógeno mais frequentemente detectado (18,9%; 7/37), ocorrendo em detecções simples e mistas (incluindo a única detecção tripla: BCoV + *M. haemolytica* + *H. somni*). Nenhum dos 10 patógenos investigados foi detectado em 62,2% (23/37) dos SNP. Os potenciais DPV-PIV agentes identificados foram *P. multocida* (8,1%; 3/37), BoAHV1, *H. somni* e *M. haemolytica* (cada um com 5,4%; 2/37). Análises exploratórias identificaram possíveis associações entre salivagem profusa e *P. multocida*, crescimento retardado e BoAHV1, e febre com BCoV. Entretanto, devido ao tamanho reduzido da amostragem, essas associações requerem confirmação em coortes maiores. No entanto, esses resultados demonstraram que os animais vacinados nesses confinamentos desenvolveram um quadro compatível com PVD-VIP e sugerem que a PVD-VIP, com manifestações clínicas compatíveis com a DRB, pode ocorrer em bovinos vacinados, apesar da imunização contra os agentes da DRB.

TERMOS DE INDEXAÇÃO: Alfa herpesvírus bovino 1; coronavírus bovino; doença respiratória bovina; padrões de detecção; *Histophilus somni*; modelagem de infecção; *Mannheimia haemolytica*; detecção molecular.

INTRODUCTION

The bovine respiratory disease (BRD) complex is a multi-etiological entity caused by a wide range of bacterial and viral pathogens associated with abrupt changes in climatic and environmental conditions as well as management practices (Caswell et al. 2012; Castro et al. 2021; Adekunle & Kaniyamattam 2026). Brazil is currently the world's largest beef producer (20%) and, together with the USA (19%), Australia (5%), and Canada (2%), produces approximately 46% of all beef consumed worldwide in 2025 (USDA-FAS 2026). However, there remains a paucity of data from Brazil regarding the occurrence and geographical distribution of BRD-related pathogens, diagnostic strategies, host-pathogen relationships, epidemiological data, and various aspects of bovine pulmonary disease when compared with data produced from the USA, Canada, and Australia. Nonetheless, most known BRD-related pathogens have been diagnosed in cattle from Brazil (Castro et al. 2021), profitability at feedlots was estimated at around 130 days-on-feed (Madeira et al. 2026), and the economic cost of BRD at feedlots within this continental nation was projected to be approximately 4 million USD annually (Baptista et al. 2025; Madeira et al. 2026). Additionally, the development of a quantitative real-time PCR platform for the detection of BRD pathogens will substantially reduce the cost of pathogen diagnosis and the turnaround time between sampling and pathogen detection (Rodriguez et al. 2026). Therefore, significant efforts are being made to reduce the disparity between Brazil and other major cattle-producing nations in obtaining local information pertaining to BRD.

BRD-related pathogens induce a wide-range of clinical manifestations in the affected animal (Griffin et al. 2010; Fulton 2020; Pardon & Buczinski 2020). These clinical signs may be associated with the intensity and infectivity of a specific pathogen (Griffin 1998), the efficiency of the host defense mechanisms and the virulent effects of each pathogen (Gershwin et al. 2015). The clinical diagnosis of BRD at feedlots is very challenging, which has resulted in the utilization of several scoring systems (Kamel et al. 2024; Adekunle & Kaniyamattam 2026), with the objectives of standardizing interpretations at feedlot operations. However, the accuracy of these scoring systems is related to the training of pen-checkers, which may frequently result in false-positives (Timsit et al. 2016). Since no scoring system to detect BRD was specifically designed for feedlot conditions in Brazil, the modified DART system (Step et al. 2008), is frequently used to evaluate respiratory discomfort at feedlots within this continental nation (Baptista et al. 2017; Magalhães et al. 2017; Madeira et al. 2026).

Understanding the association between clinical manifestations of respiratory disease and pathogen detection is fundamental to clarifying the complex relationships involved in BRD. These relationships may involve simultaneous infections with a mixture of viral and bacterial pathogens, in which viral agents commonly induce immunosuppression with consequent proliferation of commensal bacteria (Gaudino et al. 2022). With this in mind, numerous studies from Brazil (Oliveira et al. 2020b; Frucchi et al. 2022; Frucchi et al. 2024; Pedroso et al. 2026) and elsewhere (Ng et al. 2015; Paller et al. 2017; Buczinski et al. 2024) have demonstrated differences in the occurrence of infectious BRD pathogens between asymptomatic and sick cattle. Moreover, the nasal microbiome of cattle has been shown to be important in determining the effects of BRD (Centeno-Martinez et al. 2022; Centeno-Delphia et al. 2025), with differences identified between the nasal microbiome of asymptomatic and BRD-affected animals (Centeno-Martinez et al. 2022). It is worth mentioning that initial analyses suggest that specific BRD viral pathogens can be detected via either the nasal or oral cavity (Perotta et al. 2025).

Several studies have correlated the clinical manifestation of BRD with pathogen detection. An investigation from Southeastern Brazil reported a direct association between purulent nasal discharge and infections by *Mollicutes* in dairy calves (Gaeta et al. 2018). While *Mycoplasma* (*Mycoplasma*) *bovis* was the only pathogen associated with clinical BRD in Canada (Francoz et al. 2015). Alternatively, a Dutch study reported no association between pathogen detection and clinical manifestations of BRD (Antonis et al. 2022). A review of the major data bases revealed that studies from Brazil that investigated the possible associations of clinical manifestations of respiratory discomfort and pathogen detection as well as the possible roles of vaccination in BRD are lacking. Consequently, the objectives of this study were: a) to identify possible BRD pathogens, detection patterns, and occurrence of mixed infections; b) to investigate possible associations between specific clinical manifestations and pathogen detection, and c) to identify the possible post-vaccination detection of vaccine-included pathogens (PVD-VIP) in vaccinated cattle at three feedlots from Brazil.

MATERIALS AND METHODS

Ethical approval. Approval was granted by the Animal Ethics Committees for Animal Usage of the Universidade Norte do Paraná (CEUA, UNOPAR; protocol #008/20; February/2022).

Study locations, animals, and sampling. The cattle involved in this study were maintained at three beef feedlots within the states of São Paulo (SP), Mato Grosso (MT), and Goiás (GO). In 2023, the state of MT had the largest cattle herd in Brazil and contributed 14.3% (34,075,004/238,620,910) of all cattle reared in the country (IBGE 2023). Additionally, the state of GO contained the third largest cattle herd (9.9%; 23,729,878/238,620,910), while the state of SP contributed 4.5% (10,763,360/238,620,910) of all cattle reared in Brazil (IBGE 2023). Feedlot A, from the municipality of Bebedouro, SP, was in Southeastern Brazil, whereas feedlot B, from the municipality of Lucas do Rio Verde, MT, and feedlot C, in Jussara, GO, were both located in the Midwestern region of the country. Furthermore, all feedlots were located within the Cerrado biome of Brazil. Feedlot A had a holding capacity of 9,000 head of cattle, while the capacities of feedlots B and C were 17,000 and 35,000 head of cattle, respectively. The age of all steers ranged from 24 to 36 months.

Deep nasopharyngeal swabs were collected from 37 steers distributed among the three feedlots as follows: Goiás, 10 animals; Mato Grosso, 14 animals; and São Paulo, 13 animals. Animals were selected during routine pen inspection approximately 60–80 days after feedlot entry. Steers with clinical manifestations compatible with BRD were identified by experienced pen-checkers using the modified DART system (Step et al. 2008) and clinical signs of sickness in cattle (Ferraro et al. 2021), whereas asymptomatic steers were selected from the same feedlot populations when no respiratory or systemic clinical signs were observed at the time of sampling. The sampled animals included both clinically affected and asymptomatic steers from the feedlots, and their clinical status was recorded before molecular testing. Sampling was done approximately between 60–80 days after entry at these feedlots. All samples were immediately placed on ice and then maintained at -80°C until used in molecular assays.

The clinical signs recorded included profuse salivation, cough, apathy, nasal secretion, stunted growth, fever, diarrhea, and dehydration. All clinical data obtained were recorded and included in an Excel spread sheet and then used as input for the statistical analyses.

Molecular detection of BRD pathogens. The nucleic acids from all DNS were extracted with the automated extraction system Loccus Extracta 32 (Loccus do Brasil Ltda. São Paulo, Brazil) using the commercial DNA extraction kit (Extracta Kit DNA and RNA pathogens MDX, Loccus do Brasil), as recommended by the manufacturer, and then used in molecular assays designed to amplify the DNA/RNA of 10 pathogens associated with BRD. These pathogens included BVDV, BoAHV1, bovine parainfluenza virus 3 (BPIV3), bovine coronavirus (BCoV), bovine respiratory syncytial virus (BRSV), ovine gammaherpesvirus 2 (OvGHV2), *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma mycoides* (*Mycoplasma*) *bovis*. A list of the target genes and the amplicon sizes of the agents used during this investigation is provided (Supplementary Table 1). Positive controls consisted of the utilization of nucleic acids of these agents derived from previous investigations (Headley et al. 2023a; Headley et al. 2023b). The negative control consisted of ultrapure water. Positive and negative controls were included in each molecular assay.

All PCR products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide and examined under ultraviolet light. The products derived from all molecular assays were purified using the PureLink® Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen® Life Technologies, Carlsbad, CA, USA), quantified by using a Qubit® Fluorometer (Invitrogen® Life Technologies, Eugene, OR, USA), and submitted to direct sequencing in both directions with the forward and reverse primers used in the respective molecular assays in an ABI3500 Genetic Analyzer sequencer with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®, Foster City, CA, USA).

Sequence quality analyses and consensus sequences were obtained using PHRED and CAP3 (<http://asparagin.cenargen.embrapa.br/phph>), respectively. Similarity searches of the PCR products obtained were performed with nucleotide (nt) sequences deposited in GenBank using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Vaccination status, determination of pathogen circulation, and identification of post-vaccination detection of vaccine-included pathogens. All steers at these feedlots were immunized, via subcutaneous administration, with an inactive commercial respiratory vaccine containing *H. somni*, *P. multocida*, *M. haemolytica*, BoAHV1, BPIV3, and BVDV. Immunization of all animals was done at feedlot entry; only one dose was administered as recommended by the manufacturer. All DNS were collected from the steers approximately after 60-80 days-on-feed.

An animal was considered “pathogen-positive” when the target agent was detected by one of the molecular assays described above. For each pathogen evaluated, prevalence was calculated as the number of positive animals divided by the total number of vaccinated animals ($n = 37$) and expressed as a percentage. Since all animals were vaccinated and a non-vaccinated control group was not available, vaccine effectiveness could not be estimated. Accordingly, these results were interpreted as evidence of pathogen circulation and potential post-vaccination detection of vaccine-included pathogens (PVD-VIP). Additionally, a positive molecular result was interpreted as detection of nucleic acid of a given pathogen in DNS and not, by itself, as confirmation of active infection or causation of clinical disease.

Statistical analysis. Descriptive and analytic statistical methods were used to analyze the data contained in the Excel spread sheet. All analytical statistical analyses were done with the R software (version 4.4.2) with the “nnet” package for multinomial logistic regression and the “brglm2” package for bias-reduced estimation.

Statistical analyses were done utilizing a combination of multivariate (comparing multiple outcomes), and multivariable (e.g., comparing single clinical outcomes with multiple pathogens) regressions with multinomial logistic models (cluster analysis via single, dual and triple detections) to investigate the possible relationships between the clinical signs observed in feedlot cattle and categories of pathogen detection (not detected, singular, dual, and triple). Initially, a multivariate analysis incorporating Wald test statistics and PIT-trap resampling (999 iterations) was done to examine the overlapping relationships between clinical signs and detection status, while considering the potential correlation among response variables. Variables with $p \leq 0.05$ were classified as statistically significant, whereas $p \leq 0.10$ was classified as marginally significant.

Subsequently, multinomial logistic regression analysis was done by using the “Not detected” group as the reference category to delineate predictors correlated with various levels of pathogen detection (singular, dual, and triple). Due to the presence of quasi-complete separation within the dataset (as indicated by unstable standard errors and convergence warnings), bias-reduced estimation through the brglmFit method was applied to obtain stable parameter estimates and to facilitate reliable statistical estimates.

Regression coefficients (β) along with corresponding p-values were used to assess the strength and statistical significance of the associations between clinical predictors (profuse salivation, cough, apathy, nasal secretion, stunted growth, fever, diarrhea, and dehydration) and the pathogen detection categories. The threshold for statistical significance was established at $p \leq 0.05$.

RESULTS

Molecular identification of BRD pathogens in DNS. The molecular assays detected the desired amplicons for six of the 10 pathogens investigated, resulting in the amplification of four bacterial (*M.*

haemolytica, *P. multocida*, *H. somni*, and *M. bovis*) and two viral (BCoV and BoAHV1) agents of BRD. Direct sequencing confirmed the molecular detections. Selected sequences were deposited in GenBank (BCoV/BRA-UEL/653-28/2023; GenBank PZ418332). Additionally, the nucleic acids of OvGHV2, BVDV, BRSV, and BPIV3 were not detected in any of the DNS evaluated.

Epidemiological data of pathogen detection in feedlots. The distribution of the pathogen detection patterns observed at the three feedlots is graphically represented at Figure 1 and summarized in Table 1, with singular, dual, and triple detections observed at these feedlots. Although there was no statistical difference between the types of pathogen detection at the feedlots, singular pathogen detection (24.3%; 9/37) was more frequently identified relative to dual (10.8%; 4/37) and triple (2.7%; 1/37) pathogen detection (Figure 2). BCoV and *P. multocida* were the most frequently identified pathogens (Figure 2) in singular detections (8.1%; 3/37). However, BCoV was the most frequently identified pathogen (18.9%; 7/37) in all detection patterns observed during this study. Additionally, none of the 10 pathogens evaluated were detected in 62.2% (23/37) of the steers evaluated.

Although the pathogens evaluated were more frequently detected in sick cattle in comparison with asymptomatic animals at the three feedlots (Figure 3), there was no statistically significant association between the detection of pathogens in animals from these two groups. Singular pathogen detection occurred in animals from all three feedlots and included *P. multocida*, *M. bovis*, BCoV, and BoAHV1 (Figure 4). Several dual pathogen detections were identified, with a combination of viral and bacterial agents occurring in most of these. These dual detections consisted of the following: a) BCoV and *P. multocida* in the feedlot from SP; b) BoAHV1 and *M. haemolytica* in MT; and c) BoAHV1 and BCoV occurred in the feedlots from MT and GO. Furthermore, the only triple pathogen detection (BCoV, *M. haemolytica*, and *H. somni*) identified occurred in one animal from MT (Figure 4).

Associations between pathogen detection and clinical manifestations of BRD. The association between the pathogen detection and the clinical manifestations observed is summarized in Table 2 and graphically presented at Figure 5. The multivariate model (Table 2) identified profuse salivation and stunted growth as statistically significant predictors of pathogen detection frequency ($p < 0.05$). Additionally, nasal secretion and fever had borderline associations ($p < 0.10$). However, cough, apathy, diarrhea, and dehydration were not significantly associated with the frequency of pathogen detection. The multinomial logistic regression analysis was done using the not detected group as the reference category. For the singular pathogen detection category, profuse secretion ($\beta = -19.41$, $p < 0.001$), cough ($\beta = 34.87$, $p < 0.001$), nasal secretions ($\beta = -44.75$, $p < 0.001$), stunted growth ($\beta = -6.38$, $p < 0.001$), fever ($\beta = 36.48$, $p < 0.001$), diarrhea ($\beta = -3.40$, $p < 0.001$), and dehydration ($\beta = 32.40$, $p < 0.001$) were significantly associated with detection status. However, apathy ($\beta = 20.22$, $p = 0.56$) was not significantly associated with singular pathogen detection.

Cluster analyses demonstrated several significant associations between dual pathogen detection and clinical signs; these included profuse salivation ($\beta = -20.34$, $p < 0.001$), cough ($\beta = -15.63$, $p < 0.001$), nasal secretions ($\beta = -43.77$, $p < 0.001$), stunted growth ($\beta = -58.78$, $p < 0.001$), and fever ($\beta = -16.12$, $p < 0.001$). However, diarrhea and dehydration could not be estimated for the dual pathogen detection due to the sparse information contained in the dataset. Similarly, all clinical predictors were significantly associated with the triple pathogen detection, including profuse salivation ($\beta = -2.83$, $p < 0.001$), cough ($\beta = -4.45$, $p < 0.001$), apathy ($\beta = 1.49$, $p < 0.001$), nasal secretion ($\beta = -85.74$, $p < 0.001$), stunted growth ($\beta = -25.42$, $p < 0.001$), fever ($\beta = 37.37$, $p < 0.001$), diarrhea ($\beta = 0.82$, $p < 0.001$), and dehydration ($\beta = 1.53$, $p < 0.001$), demonstrating clinical differentiation between the animals with and without triple pathogen detection.

Single pathogen-clinical analysis revealed significant associations between pathogen detection and the clinical manifestations of BRD; these included: a) profuse salivation with the detection of *P. multocida* ($\beta = 2.30$, SE = 1.17, $p = 0.049$); b) stunted growth with BoAHV1 ($\beta = 2.30$, SE = 1.17, $p = 0.049$); and c) fever with BCoV ($\beta = 2.98$, SE = 1.16, $p = 0.010$). These associations are graphically illustrated in Figure 5. Although there was a positive correlation between the occurrence of cough and the detection of *P. multocida*, this association was not statistically significant ($\beta = 1.98$, SE = 1.13, $p = 0.08$). Furthermore, all additional associations identified between pathogen detection and clinical manifestations of respiratory discomfort had no statistically significant ($p > 0.05$) association.

Identification of post-vaccination detection of vaccine-included pathogens. All 37 animals in the study received a commercial multivalent vaccine against common respiratory pathogens of ruminants, including *H. somni*, *P. multocida*, *M. haemolytica*, BoAHV1, BPIV3, and BVDV. Despite this immunization protocol, several BRD agents were detected in cattle that had been previously vaccinated, resulting in post-vaccination detection of vaccine-included pathogens (PVD-VIP). These PVD-VIP-associated BRD pathogens and their related prevalence are summarized in Table 3 and included *P. multocida* in 8.1% (3/37) of all animals, while the PVD-VIP prevalence of BoAHV1, *H. somni*, and *M. haemolytica* was 5.4% (2/37).

Although OvGHV2, BRSV, BCoV, and *M. bovis* were not included in the commercial vaccine used at these feedlots, BCoV and *M. bovis* were identified in this dataset, resulting in detection rates of 18.9% (7/37) and 2.7% (1/37), respectively.

DISCUSSION

The clinical recognition of BRD under feedlot conditions remains challenging, and the performance of scoring systems depends heavily on observer training, which can contribute to misclassification and the detection of false positives (Timsit et al. 2016; Adekunle & Kaniyamattam 2026). Since a scoring system developed for Brazilian feedlots is lacking, the application of internationally recognized adapted methods (e.g., modified DART) is common in Brazil (Baptista et al. 2017; Magalhães et al. 2017; Madeira et al. 2026). These constraints reinforce the importance of combining clinical observations with infectious disease diagnostics and analytical approaches capable of separating overlapping clinical signs and heterogeneous detection states (Caswell et al. 2012; Pardon & Buczinski 2020). Accordingly, this study used a combination of recommended clinical observations coupled with molecular diagnostics and statistical analyses to investigate the associations herein described. Although the results herein obtained were derived from a small population of animals from three distinct geographical regions of Brazil, these initial findings are fundamental and can serve as a basis for the understanding of pathogen detection dynamics in feedlot cattle from Brazil. Additionally, this is one of the relatively few studies from Brazil that investigated possible associations between BRD-clinical status and pathogen detection, since only one previous study (Gaeta et al. 2018) was identified when major English and Latin databases (PubMed, Scopus, Google, and Scielo) were searched.

The clinical manifestations observed in these animals are well-known signs of BRD and clinical manifestations of diseases in cattle worldwide (Buczinski et al. 2014; Francoz et al. 2015; Ferraro et al. 2021). The results of this study have demonstrated that cattle at these feedlots developed clinical manifestations suggestive of BRD despite being immunized with a commercial vaccine at feedlot entry, resulting in potential post-vaccination detection of vaccine-included pathogens (PVD-VIP) respiratory infections by specific pathogens. The PVD-VIP in BRD, as herein detected, may occur when calves or cattle become clinically ill despite having pre-existing immunity (vaccination, maternal antibodies, or prior exposure); similar detection of BRD pathogens in vaccinated cattle was reported in Brazil (Magalhães et al. 2017; Fritzen et al. 2024) and elsewhere (Zhang et al. 2019; Hegazy et al. 2025). Furthermore, during this study the nucleic acids of the 10 tested pathogens were not detected in a substantial proportion of animals at the time of sampling. Since all animals were vaccinated, it may suggest there was reduced exposure to these BRD pathogens, considering that 62.2% (23/37) of the animals did not contain any of the 10 pathogens investigated. In addition, the non-detection of the nucleic acids of OvGHV2, BVDV, BRSV, and BPIV3 may suggest that these pathogens were not detected at the time of sampling.

The present investigation demonstrated that, although singular, dual, and triple detection patterns were detected, singular detections were the most frequent. The identification of more single-pathogen detections than simultaneous infections is frequently described in cattle from different geographical regions of Brazil (Headley et al. 2018; Oliveira et al. 2020a; Frucchi et al. 2024; Perotta et al. 2025; Rodriguez et al. 2026) as well as in other countries, including the USA (Gershwin et al. 2015), UK (Denholm et al. 2024), Canada (Buczinski et al. 2024), Ireland (Murray et al. 2017), France (Dorso et al. 2021), and China (Zhou et al. 2023). These findings may suggest that most respiratory infections in cattle are more frequently associated with the occurrence of only one pathogen, whereas the detection of concomitant agents demonstrates the multitietiological nature of BRD.

In addition to the singular pathogen detections herein observed at these feedlots, there was the concomitant detection of pathogens (dual and triple) in which combinations of viral and bacterial agents were identified as well as the simultaneous detection of viral pathogens (BoAHV1 and BCoV) in the feedlots from Goiás and Mato Grosso. Similar dual and triple pathogen detections were previously described in feedlots from the state of Paraná, Southern (Headley et al. 2018), and MG, Midwestern (Baptista et al. 2017) Brazil. Moreover, similar patterns of pathogen detection were observed in feedlot cattle from the USA (Fulton et al. 2009), Canada (Gagea et al. 2006), and France (Padalino et al. 2021). Collectively, the patterns of pathogen detection (singular, dual, and triple) observed in this study are aligned with the detection patterns described worldwide. The detection of dual or triple infections containing viral and bacterial pathogens herein demonstrates the immunosuppressive actions of most BRD viral pathogens with subsequent proliferation of commensal bacteria after the disruption of the local immune system of the affected host (Cusack et al. 2003; Chai et al. 2022; Gaudino et al. 2022). This mechanism continues to be the hallmark of infections by viral pathogens, contribute meaningfully to the understanding of BRD pathogenesis, alters bacterial colonization within the lungs and disease expression (Righi et al. 2023). Furthermore, most of the bacterial pathogens herein detected (*H. somni*, *M. haemolytica*, and *P. multocida*), are frequently identified in feedlot cattle acting as commensal or secondary agents (Fulton et al. 2009; Adekunle & Kaniyamattam 2026), and their detection contributes towards the dynamic multitietiological nature of BRD. Alternatively, the possibility of *M. bovis*, herein identified, acting as a primary or secondary agent of BRD remains controversial (Oliveira et al. 2020a), considering the global detection of this pathogen in multiple organs, farming, and production systems (Su et al. 2025), and the complexity of the immune mechanisms of this microorganism (Zhang et al. 2022). Additionally, the non-identification of statistical differences between the detection of BRD pathogens in sick and asymptomatic animals during this study may reflect the dataset. This is because in other studies, with a larger number of animals there were statistical differences in the occurrence of pathogen detection in sick and asymptomatic cattle (Francoz et al. 2015; Ng et al. 2015; Magalhães et al. 2017; Paller et al. 2017; Frucchi et al. 2024).

No statistical difference was detected between the positive detection of these pathogens at the feedlots investigated from three distinct geographical locations within the same biome of Brazil. These results are contrary to a recent study that demonstrated that feedlot cattle maintained within the state of MT had more elevated risk of developing pulmonary disease as compared to cattle reared in feedlot from the state of Minas Gerais, Southeastern Brazil (Madeira et al. 2026). Therefore, caution must be taken with the interpretation of these specific results from the current study considering the reduced number of cases herein evaluated.

It must be highlighted that molecular assays confirmed the detection of pathogen in the anatomical location investigated, but they do not necessarily distinguish between transient contamination, colonization/carriage, active infection, or the direct cause of clinical disease. This distinction is particularly important for bacterial agents associated with BRD, such as *P. multocida*, *M. haemolytica*, *H. somni*, and *M. bovis*, which may colonize the upper respiratory tract of cattle and act as commensal, opportunistic, or secondary pathogens under specific host, environmental, or viral-immunosuppressive conditions (Cusack et al. 2003; Castro et al. 2021; Chai et al. 2022). Therefore, the significant associations observed herein should be interpreted as molecular detection patterns associated with clinical manifestations, rather than definitive evidence that the detected agent was the primary cause of the corresponding clinical sign.

Post-vaccination detection of vaccine-included pathogens may occur frequently in vaccinated feedlot cattle. An interesting finding during this investigation was the detection of several pathogens contained in the commercial vaccine administered at these feedlots which we termed post-vaccination detection of vaccine-included pathogens. This phenomenon herein observed at these beef cattle feedlots is referred to as breakthrough infection (BTI) in human medicine (Mohseni Afshar et al. 2022). Accordingly, a potential a BTI infection or post-vaccination detection of vaccine-included pathogens (PVD-VIP) in BRD may be defined as the development of clinical respiratory disease with pathogen detection in cattle that occurred after vaccination, maternal immunity, or previous pathogen exposure should have provided protection, but was overcome by a combination of several mechanisms including pathogen evasion, antigenic drift, coinfection synergy, or incomplete immune response. Accordingly, the simultaneous detection herein described of several BRD pathogens contained within this commercial vaccine, in vaccinated cattle from these three feedlots which had clinical manifestations of respiratory disease fits this definition and classification.

During this study three bacterial pathogens (*M. haemolytica*, *P. multocida*, and *H. somni*) and BoAHV1 were identified as producing potential PVD-VIP events at these feedlots after routine entry vaccination. An experimental study from Midwestern Brazil, detected only *H. somni* (5.6%) and *M. haemolytica* (16.2%) in 37 vaccinated feedlot cattle immunized with a commercial vaccine that contained four viral BRD agents (Magalhães et al. 2017). Additionally, dairy cattle from Southern Brazil with clinical manifestations of BRD and immunized with a commercial BVDV vaccine were infected with BVDV-2b, not contained in the immunogen, as well as with *P. multocida* and *M. haemolytica* (Fritzen et al. 2024). Moreover, BVDV, along with BCoV, BRSV, and *M. bovis* were detected within the nasopharynx-associated lymphoid tissue of calves from Japan vaccinated against BVDV (Hegazy et al. 2025), while metagenomic analyses in an experimental study from Alberta, Canada, detected a wide-range of pathogens, including BRSV, BVDV, and BoAHV1, in calves that received two doses of modified-live vaccines containing these BRD-agents (Zhang et al. 2019). Furthermore, *M. haemolytica* (48%) and *H. somni* (26%) were detected in calves immunized at feedlot entry in Alberta, Canada, with vaccines containing these pathogens (Guo et al. 2020). Collectively these studies demonstrated that the control of BRD pathogens in feedlots is very complicated, not straightforward, and that PVD-VIP at beef cattle feedlots may be a frequent occurrence.

The mechanisms associated with PVD-VIP in cattle with respiratory disease are complex, as a result a detailed discussion of these mechanisms is beyond the scope of this paper. These mechanisms affect the host-pathogen-environment homeostasis of disease and may include viral immune suppression (Gaudino et al. 2022; Ostler & Jones 2023; Righi et al. 2023), bacterial evasion (Perez-Casal 2020), antigenic variation (Falkenberg et al. 2021), synergism between bacterial and viral pathogens (Lion et al. 2021; Chai et al. 2022), stress-induced conditions (Cusack et al. 2003; Smith 2020), and the interference of maternal antibody (Fulton et al. 2004; Smith 2020). In the current study, the PVD-VIP detection of BoAHV1 can be directly attributed to the immunosuppressive effects of this pathogen that frequently results in impaired cell-mediated immunity, disruption of pulmonary epithelium, and downregulation of infected T-lymphocytes (Ostler & Jones 2023). Additionally, *H. somni* herein identified as potential PVD-VIP, has a reported 32.5x more elevated risk of been detected in cattle immunized with commercial vaccine containing this pathogen 40 days after feedlot entry as compared to calves that entered 20 days previously (Timsit et al. 2017); this phenomenon may explain the detection of this pathogen as potential PVD-VIP during this study, since sampling was done approximately 70 days after feedlot entry. Therefore, it seems that longer periods in feedlots may facilitate the proliferation of *H. somni* in the affected animal or feedlots may serve as potential sources of ongoing *H. somni* infections (Guo et al. 2020).

Bovine coronavirus remains comparatively infrequently reported in respiratory disease studies from Brazil. BCoV and *P. multocida* were the most frequently occurring pathogens detected in this study, particularly in singular detections, while BCoV was identified in multiple detection categories resulting in 18.9%

(7/37) of all infections diagnosed. Alternatively, two animals from this study were infected by BoAHV1. Although these two viral pathogens are major causes of BRD worldwide (Cusack et al. 2003; Fulton et al. 2009; Adekunle & Kaniyamattam 2026), there is a comparative reduced detection of BCoV relative to BoAHV1 in cattle from Brazil with respiratory disease. Although the actual reason for the comparatively elevated detection of BCoV in this study, relative to other pathogens herein identified, is unknown, we hypothesize that this may partially be attributed to the absence of this pathogen in the commercial immunogen used at these feedlots. This is because the prevalence of the PVD-VIP agents (see below) herein identified was comparatively reduced with relation to that of BCoV.

Nonetheless, BCoV is a major pathogen associated with the development of respiratory (Cusack et al. 2003; Fulton et al. 2009) and enteric (Saif 2010; Vlasova & Saif 2021) cattle diseases worldwide but with restricted detection in cattle with respiratory impairment from Brazil. The first reported identification of BCoV in Brazil occurred after detection in clinical samples of cattle with diarrhea (Brandão et al. 2002), which was then confirmed by isolation (Jerez et al. 2005). Since then, there have been relatively few documentations of BCoV with respiratory disease in this continental nation. The association of BCoV with respiratory impairment in cattle from Brazil, based on published papers (Fig. 6), can be classified into four groups: a) isolation and molecular characterization (Jerez et al. 2005; Beuttemuller et al. 2017; Mello et al. 2024); b) concomitant infections with *M. bovirhinis* (Frucchi et al. 2024; Headley et al. 2024); c) epidemiological findings with coinfections in feedlot and dairy cattle (Headley et al. 2018; Oliveira et al. 2020b; Frucchi et al. 2022; Pedroso et al. 2026); and d) concomitant viral infections (Fritzen et al. 2024). Therefore, there are relatively few reports of BCoV-related respiratory detections in cattle from Brazil as compared to other geographical regions, including Europe (Berge & Vertenten 2024) and North America (Fulton 2009, 2020). Alternatively, serological evidence of the circulation of BoAHV1-associated BRD in Brazil varies between 43 (Santos et al. 2025) to 92% (Chicoski et al. 2023). Furthermore, BoAHV1 is one of the pathogens frequently detected in cattle with respiratory disease in this county (Castro et al. 2021).

Finally, the actual reasons for the under- or reduced diagnosis of BCoV in BRD from Brazil are not totally elucidated but may be related to several issues, including a) focus of diagnostics surveillance, considering that most of the studies with this pathogen from Brazil were related to enteric detection and characterizations; b) the ill-defined clinical and pathological lesions/syndromes due to the viral strain, age of the infected animal, and the frequent occurrence in coinfections (Vlasova & Saif 2021), can induce misdiagnosis; c) the reduced or inconsistent inclusion of this pathogen in diagnostic panels for BRD in Brazil; and d) the sporadic viral shedding in subclinically infected animals (Saif 2010), may affect identification and consequently viral detection.

Associations between clinical manifestations and pathogen detection. This is probably one of the few studies from Brazil to effectively associate the clinical manifestations of BRD with pathogen detection. Multivariable modelling identified profuse salivation and stunted growth as statistically significant predictors of detection frequency, while nasal secretion and fever showed borderline associations. These findings support the concept that clinical expression may vary with detection status, while also reinforcing that clinical diagnosis in BRD is not straightforward, since clinical signs alone are unreliable predictors for specific pathogens, with low sensitivity and frequent subclinical disease (Ferraro et al. 2021). At the pathogen-clinical sign level, statistically significant associations were identified between profuse salivation and the detection of *P. multocida*, stunted growth with BoAHV1, and fever with BCoV. These results are in accord with investigations that have demonstrated specific relationships between pathogen detection and definite clinical signs of BRD in diverse geographical locations. An Italian study demonstrated that cattle with nasal discharges had an increased risk of having *M. haemolytica* detected (Padalino et al. 2021); the detection of *M. bovis* was associated with reduced average daily gain in Canada (Francoz et al. 2015); while purulent nasal discharges were related to the detection of *Mollicutes* in a study from São Paulo, Brazil (Gaeta et al. 2018).

Collectively, these results underscore that some clinical signs may be more informative under specific epidemiological and geographical conditions, but that many commonly observed signs of respiratory disease may have limited discriminatory value when used in isolation for the detection of specific BRD pathogens (Timsit et al. 2016; Kamel et al. 2024). It must be highlighted that although there was statistical significance for the associations between some clinical signs and pathogen detection, an adequate understanding of these specific associations would require a more extensive and robust dataset. Furthermore, as indicated previously, some studies have demonstrated associations between clinical signs and pathogen detection (Francoz et al. 2015; Gaeta et al. 2018; Padalino et al. 2021), while others have established no definite relationship (Antonis et al. 2022). Additionally, the multinomial logistic regression model further supported clinical differentiation across detection categories (not detected, singular, dual, triple), while also illustrating analytic challenges that can arise in BRD datasets when observations are sparse in higher-order detection groups. Despite these constraints, the use of bias-reduced estimation was necessary to obtain stable estimate given quasi-complete separation and unstable standard errors. Collectively, these results reinforce the importance of combining systematic clinical diagnosis, molecular testing, with appropriately selected statistical analyses when evaluating the heterogeneous presentations that are characteristic of BRD (Kamel et al. 2024; Adekunle & Kaniyamattam 2026). Nevertheless, a larger database will be needed to understand the possible associations between clinical signs of BRD and

pathogen detection in cattle from Brazil. Moreover, BRD is a dynamic syndrome in which pathogen shedding, host immune responses, and clinical manifestations may vary over time. Consequently, some animals may have shown clinical signs after earlier pathogen exposure that was no longer detectable, whereas others may have harbored detectable nucleic acids without active disease at the time of sampling. This temporal uncertainty and the small data set reinforces the need for cautious interpretation of the observed associations and supports the use of longitudinal designs and quantitative molecular approaches in future studies.

Study limitations and future perspectives. This study has several limitations that should be considered when interpreting the present findings. Firstly, the sample size was relatively small ($n = 37$), which restricted statistical association, particularly for higher order detection categories such as dual and triple pathogen detections. This limitation was reflected in the presence of quasi complete separation and unstable parameter estimates in multinomial regression analyses, necessitating the use of bias reduced estimation techniques. While this approach allowed meaningful estimate, larger datasets would have enabled a more robust estimation and improved generalizability of results. Nonetheless, the results herein described are important in understanding the dynamics of pathogen detection and/or infections and possible PVD-VIP at beef cattle feedlot in Brazil, considering the scarcity of related data from the major beef producer. Expanding the sample size and including diverse production systems and regions of Brazil would enhance statistical robustness and allow more precise estimation of mixed detection dynamics.

Secondly, the study adopted a cross-sectional design with a single sampling time point, approximately 60-80 days after feedlot entry. Bovine respiratory disease is a dynamic process characterized by transient pathogen shedding, fluctuating immune responses, and evolving clinical manifestations. Consequently, pathogen detection at a single point in time may not fully capture earlier infection/detection events or the temporal sequence linking pathogen exposure to disease expression. This temporal limitation could have contributed to the relatively high proportion of animals with no detectable pathogens despite recorded clinical signs. Furthermore, the utilization of quantitative molecular diagnostics to assess pathogen load, rather than presence or absence alone, would have facilitated the differentiation between passive carriage and clinically relevant detection, and assist with the interpretation of the associations between pathogen detection and BDR-signs.

Thirdly, the evaluation of clinical manifestations of disease by pen-checkers could have resulted in the nonidentification or over representations of the signs of disease, since the effectiveness of this service is directly related to the training received and the experience by these. Additionally, molecular detection from deep nasopharyngeal swabs should not be interpreted as definitive evidence of active infection or direct disease causation, since bacterial BRD-associated agents may also represent transient colonization or carriage within the upper respiratory tract, particularly in the absence of quantitative pathogen-load data, lower-respiratory tract samples, or longitudinal follow-up.

Fourthly, no formal correction for multiple comparisons was applied to the several clinical sign-pathogen detection associations evaluated in this study; consequently, the possibility of statistical error cannot be excluded, and associations with borderline p-values should be interpreted cautiously as hypothesis-generating rather than definitive evidence of pathogen-specific clinical relationships. Additionally, the small number of animals in the dual and triple pathogen detection categories reduced the robustness of the corresponding model estimates. Although bias-reduced estimation was applied to address quasi-complete separation, these sparse-data estimates should be interpreted cautiously as exploratory and hypothesis-generating, with confidence intervals used to reflect their uncertainty.

Finally, all animals evaluated were vaccinated, and a non-vaccinated comparison group was not available. While pathogen detection in vaccinated cattle was interpreted as evidence of pathogen circulation and potential breakthrough infections, vaccine effectiveness could not be estimated, and bacterial resistance was not the objective of this investigation. As a result, conclusions regarding vaccination outcomes must be interpreted cautiously and within the context of partial immunity rather than vaccine failure. Notwithstanding the above, the information contained in this manuscript is important and fundamental for the local cattle industry and may serve as the basis for the evaluation of BTI not only in beef cattle but also in dairy herds from Brazil.

Future investigations should prioritize longitudinal study designs that track cattle from feedlot entry through finishing, incorporating repeated sampling to better characterize pathogen detection, persistence, and clearance over time. Longitudinal data would enable clearer evaluation of temporal relationships between pathogen detection, clinical progression, and recovery, as well as improved identification of early predictors of disease onset.

Future work should also integrate quantitative molecular diagnostics to assess pathogen load, rather than presence or absence alone. Quantitative data may be able to differentiate between passive carriage and clinically relevant infection, aiding interpretation of associations between pathogen detection and disease severity. Coupling quantitative diagnostics with nasal microbiome analyses could further elucidate the role of microbial community structure in BRD susceptibility and resilience.

Additionally, since vaccine included agents were detected in immunized cattle, additional research is warranted to evaluate vaccination strategies, including timing, booster protocols, and antigen composition, under feedlot conditions in Brazil. Moreover, comparative studies with vaccinated and non-vaccinated cohorts,

where ethically and logistically feasible, would allow clearer estimate regarding vaccine impact on pathogen circulation and disease expression in this country.

CONCLUSION

This cross-sectional molecular survey demonstrated that BRD-associated pathogens circulated in feedlots from Brazil despite routine entry immunization with an inactivated multivalent respiratory vaccine at feedlot entry. Most animals had no detectable pathogens at the sampling point, but single-agent detections were predominant. However, BCoV was the most frequently detected pathogen and occurred in both single and mixed detection patterns. Significant associations were detected between some clinical signs (profuse salivation, stunted growth, and fever) and the detection of *P. multocida*, BoAHV1, and BCoV suggesting that combining standardized clinical scoring (modified DART) with molecular testing can improve interpretation of BRD heterogeneity under field conditions. Since vaccine effectiveness could not be estimated, the detections of vaccine-included agents (*P. multocida*, *H. somni*, *M. haemolytica*, and BoAHV1) after initial immunization demonstrated potential PVD-VIP at these feedlots. Additionally, there is the need for continued surveillance, inclusion of BCoV in diagnostic panels of BRD, and larger longitudinal studies to clarify temporal dynamics, pathogen load, and the impact of vaccination strategies on beef production systems in feedlots from Brazil.

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Data availability statement.- The partial nucleotide sequences of the BCoV and BoAHV1 strains identified during this study are deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>).

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process.- During the preparation of this work the authors used Copilot and Gamma to generate figure 6 contained in this paper. The prompts for the two figures were obtained from LeapSpace (<https://www.sciencedirect.com/leapspace>) and then generated in Gamma AI. After using this tool/service, the author(s) reviewed and edited the content as needed and took full responsibility for the content of the published article.

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REFERENCES

- Adekunle, A. & Kaniyamattam, K. 2026. Bovine respiratory disease: epidemiological drivers, transmission dynamics, and economic implications in beef production systems. *Agriculture*. 16: 311.<https://doi.org/10.3390/agriculture16030311>
- Antonis, A. F. G., Swanenburg, M., Wisselink, H. J., Smid, B., van Klink, E. & Hagenaars, T. J. 2022. Respiratory pathogens in veal calves: Inventory of circulating pathogens. *Vet Microbiol*. 274: 109571.<https://doi.org/10.1016/j.vetmic.2022.109571>
- Baptista, A. L., Rezende, A. L., Fonseca, P. d. A., Nogueira, G. M., Headley, S. A., Menezes, G. L., Alfieri, A. A. & Saut, J. P. E. 2017. Bovine respiratory disease complex associated mortality and morbidity rates in feedlot cattle from southeastern Brazil. *J Infect Dev Ctries*. 11: 791–799.<https://doi.org/10.3855/jidc.9296>
- Baptista, A. L., Xavier, A. A. C., Reis, M. H., Madeira, R. B., Cerqueira, H. D. B., Gama, R., Headley, S. A. & Saut, J. P. E. 2025. The economic impacts of the bovine respiratory disease complex on beef cattle feedlots from Brazil. *Semin Cienc Agrar*. 46: 1401–1416.<https://doi.org/10.5433/1679-0359.2025v46n5p1401>

- Berge, A. C. & Vertenten, G. 2024. Bovine coronavirus prevalence and risk factors in calves on dairy farms in Europe. *Animals*. 14: 2744. <https://doi.org/10.3390/ani14182744>
- Beuttemuller, E., Alfieri, A., Headley, S. & Alfieri, A. 2017. Bovine respiratory coronavirus Brazilian strain is derived from the dual enteric and respiratory tropism. *Genet Mol Res*. 5. <https://doi.org/10.4238/gmr16029580>
- Brandão, P. E., Birgel Jr, E. H., Gregori, F., Rosales, C. A. R., Ruiz, V. L. A. & Jerez, J. A. 2002. Bovine coronavirus detection in adult cows in Brazil. *Arquivos do Instituto Biológico*. 69: 102–104. <https://doi.org/10.1590/1808-1657v69n2p1032002>
- Buczinski, S., Broes, A. & Savard, C. 2024. Frequency of bovine respiratory disease complex bacterial and viral agents using Multiplex Real-Time qPCR in Quebec, Canada, from 2019 to 2023. *Vet. Sci*. 11: 631. <https://doi.org/10.3390/vetsci11120631>
- Buczinski, S., Forté, G., Francoz, D. & Bélanger, A. M. 2014. Comparison of thoracic auscultation, clinical score, and ultrasonography as indicators of bovine respiratory disease in preweaned dairy calves. *J Vet Intern Med*. 28: 234–242. <https://doi.org/10.1111/jvim.12251>
- Castro, M. M., Oliveira, T. E. S. & Headley, S. A. 2021. Bovine respiratory disease in Brasil: a short review. *Semin Cienc Agrar*. 42: 2081–2110. <https://doi.org/10.5433/1679-0359.2021v42n3supl1p2081>
- Caswell, J. L., Hewson, J., Slavić, Đ., DeLay, J. & Bateman, K. 2012. Laboratory and postmortem diagnosis of bovine respiratory disease. *Vet Clin North Am Food Anim Pract*. 28: 419–441. <https://doi.org/10.1016/j.cvfa.2012.07.004>
- Centeno-Delphia, R. E., Glidden, N., Long, E., Ellis, A., Hoffman, S., Mosier, K., Ulloa, N., Cheng, J. J., Davidson, J. L., Mohan, S., Kamel, M., Szasz, J. I., Schoonmaker, J., Koziol, J., Boerman, J. P., Ault, A., Verma, M. S. & Johnson, T. A. 2025. Nasal pathobiont abundance is a moderate feedlot-dependent indicator of bovine respiratory disease in beef cattle. *Anim Microbiome*. 7: 27. <https://doi.org/10.1186/s42523-025-00387-y>
- Centeno-Martinez, R. E., Glidden, N., Mohan, S., Davidson, J. L., Fernández-Juricic, E., Boerman, J. P., Schoonmaker, J., Pillai, D., Koziol, J., Ault, A., Verma, M. S. & Johnson, T. A. 2022. Identification of bovine respiratory disease through the nasal microbiome. *Anim Microbiome*. 4: 15. <https://doi.org/10.1186/s42523-022-00167-y>
- Chai, J., Capik, S. F., Kegley, B., Richeson, J. T., Powell, J. G. & Zhao, J. 2022. Bovine respiratory microbiota of feedlot cattle and its association with disease. *Vet Res*. 53: 4. <https://doi.org/10.1186/s13567-021-01020-x>
- Chicoski, L. M., Fritzen, J. T. T., Lorenzetti, E., da Costa, A. R., Moro, E., de Carvalho, E. R., Alfieri, A. F. & Alfieri, A. A. 2023. Serological profile of respiratory viruses in unvaccinated steers upon their arrival at Brazilian feedlot facilities. *Braz J Microbiol*. 54: 3237–3244. <https://doi.org/10.1007/s42770-023-01122-y>
- Cusack, P. M., McMeniman, N. & Lean, I. J. 2003. The medicine and epidemiology of bovine respiratory disease in feedlots. *Aust Vet J*. 81: 480–487. <https://doi.org/10.1111/j.1751-0813.2003.tb13367.x>
- Denholm, K., Evans, N. P., Baxter-Smith, K. & Burr, P. 2024. Retrospective study of the relative frequency of cattle respiratory disease pathogens in clinical laboratory samples submitted by UK veterinary practices. *Vet Rec*. 195: e4434. <https://doi.org/10.1002/vetr.4434>
- Dorso, L., Rouault, M., Barbotin, C., Chartier, C. & Assié, S. 2021. Infectious bovine respiratory diseases in adult cattle: an extensive necropsic and etiological study. *Animals*. 11: 2280. <https://doi.org/10.3390/ani11082280>
- Falkenberg, S. M., Dassanayake, R. P., Terhaar, B., Ridpath, J. F., Neill, J. D. & Roth, J. A. 2021. Evaluation of antigenic comparisons among bvdv isolates as it relates to humoral and cell mediated responses. *Front Vet Sci*. Volume 8 - 2021. <https://doi.org/10.3389/fvets.2021.685114>
- Ferraro, S., Fecteau, G., Dubuc, J., Francoz, D., Rousseau, M., Roy, J. P. & Buczinski, S. 2021. Scoping review on clinical definition of bovine respiratory disease complex and related clinical signs in dairy cows. *J Dairy Sci*. 104: 7095–7108. <https://doi.org/10.3168/jds.2020-19471>
- Francoz, D., Buczinski, S., Belanger, A. M., Forte, G., Labrecque, O., Tremblay, D., Wellemans, V. & Dubuc, J. 2015. Respiratory pathogens in Quebec dairy calves and their relationship with clinical status, lung consolidation, and average daily gain. *J Vet Intern Med*. 29: 381–387. <https://doi.org/10.1111/jvim.12531>
- Fritzen, J. T. T., Yasumitsu, C. Y., Silva, I. V., Lorenzetti, E., Alfieri, A. F. & Alfieri, A. A. 2024. Respiratory illness in young and adult cattle caused by bovine viral diarrhoea virus subgenotype 2b in singular and mixed bacterial infection in a BVDV-vaccinated dairy herd. *Braz J Microbiol*. 55: 4139–4146. <https://doi.org/10.1007/s42770-024-01476-x>
- Frucchi, A. P. S., Dall Agnol, A. M., Bronkhorst, D. E., Beuttemuller, E. A., Alfieri, A. A. & Alfieri, A. F. 2022. Bovine coronavirus co-infection and molecular characterization in dairy calves with or without clinical respiratory disease. *Front Vet Sci*. 9: 895492. <https://doi.org/10.3389/fvets.2022.895492>
- Frucchi, A. P. S., Dall Agnol, A. M., Caldart, E. T., Bronkhorst, D. E., Alfieri, A. F., Alfieri, A. A. & Headley, S. A. 2024. The role of *Mycoplasma bovirhinis* in the development of singular and concomitant respiratory infections in dairy calves from southern Brazil. *Pathogens*. 13. <https://doi.org/10.3390/pathogens13020114>
- Fulton, R. W. 2009. Bovine respiratory disease research (1983–2009). *Anim Health Res Rev*. 10: 131–139. <https://doi.org/10.1017/S146625230999017X>
- Fulton, R. W. 2020. Viruses in bovine respiratory disease in North America: knowledge advances using genomic testing. *Vet Clin North Am Food Anim Pract*. 36: 321–332. <https://doi.org/10.1016/j.cvfa.2020.02.004>

- Fulton, R. W., Blood, K. S., Panciera, R. J., Payton, M. E., Ridpath, J. F., Confer, A. W., Saliki, J. T., Burge, L. T., Welsh, R. D., Johnson, B. J. & Reck, A. 2009. Lung pathology and infectious agents in fatal feedlot pneumonias and relationship with mortality, disease onset, and treatments. *J Vet Diagn Invest.* 21: 464–477.<https://doi.org/10.1177/104063870902100407>
- Fulton, R. W., Briggs, R. E., Payton, M. E., Confer, A. W., Saliki, J. T., Ridpath, J. F., Burge, L. J. & Duff, G. C. 2004. Maternally derived humoral immunity to bovine viral diarrhea virus (BVDV) 1a, BVDV1b, BVDV2, bovine herpesvirus-1, parainfluenza-3 virus, bovine respiratory syncytial virus, *Mannheimia haemolytica* and *Pasteurella multocida* in beef calves, antibody decline by half-life studies and effect on response to vaccination. *Vaccine.* 22: 643–649.<https://doi.org/10.1016/j.vaccine.2003.08.033>
- Gaeta, N. C., Ribeiro, B. L. M., Alemán, M. A. R., Yoshihara, E., Nassar, A. F. C., Marques, L. M., Timenetsky, J. & Gregory, L. 2018. Bacterial pathogens of the lower respiratory tract of calves from Brazilian rural settlement herds and their association with clinical signs of bovine respiratory disease. *Pesq Vet Bras.* 38.<https://doi.org/10.1590/1678-5150-PVB-5323>
- Gagea, M. I., Bateman, K. G., van Dreumel, T., McEwen, B. J., Carman, S., Archambault, M., Shanahan, R. A. & Caswell, J. L. 2006. Diseases and pathogens associated with mortality in Ontario beef feedlots. *J Vet Diagn Invest.* 18: 18–28
- Gaudino, M., Nagamine, B., Ducatez, M. F. & Meyer, G. 2022. Understanding the mechanisms of viral and bacterial coinfections in bovine respiratory disease: a comprehensive literature review of experimental evidence. *Vet Res.* 53: 70.<https://doi.org/10.1186/s13567-022-01086-1>
- Gershwin, L. J., Van Eenennaam, A. L., Anderson, M. L., McEligot, H. A., Shao, M. X., Toaff-Rosenstein, R., Taylor, J. F., Neibergs, H. L. & Womack, J. 2015. Single pathogen challenge with agents of the bovine respiratory disease complex. *PLoS one.* 10: e0142479.<https://doi.org/10.1371/journal.pone.0142479>
- Griffin, D. 1998. Feedlot diseases. *Vet Clin North Am Food Anim Pract.* 14: 199–231.[https://doi.org/10.1016/S0749-0720\(15\)30251-6](https://doi.org/10.1016/S0749-0720(15)30251-6)
- Griffin, D., Chengappa, M. M., Kuszak, J. & McVey, D. S. 2010. Bacterial pathogens of the bovine respiratory disease complex. *Vet Clin North Am Food Anim Pract.* 26: 381–394.<https://doi.org/10.1016/j.cvfa.2010.04.004>
- Guo, Y., McMullen, C., Timsit, E., Hallewell, J., Orsel, K., van der Meer, F., Yan, S. & Alexander, T. W. 2020. Genetic relatedness and antimicrobial resistance in respiratory bacteria from beef calves sampled from spring processing to 40 days after feedlot entry. *Vet Microbiol.* 240: 108478.<https://doi.org/10.1016/j.vetmic.2019.108478>
- Headley, S. A., Dall Agnol, A. M., Alfieri, A. F., Alfieri, A. A. & Buczinski, S. 2024. The effects of simultaneous respiratory infections on the nasal shedding of *Mycoplasma bovis* in dairy calves. *Vet Res Commun.* 48: 4133–4139.<https://doi.org/10.1007/s11259-024-10529-3>
- Headley, S. A., Dall Agnol, A. M., Bessegato, J. A., Frucchi, A. P. S., Maturana É, F. L., Rodrigues, R. V., Xavier, A. A. C., Alfieri, A. F. & Alfieri, A. A. 2023a. Association of ovine gammaherpesvirus 2 with an outbreak of acute respiratory disease in dairy cattle. *Sci Rep.* 13: 5623.<https://doi.org/10.1038/s41598-023-30133-w>
- Headley, S. A., Fritzen, J. T. T., Silva, D. C., Xavier, A. A. C., Yasumitsu, C. Y., Silva, F. H. P., Alfieri, A. F., Soethe, A. M. & Alfieri, A. A. 2023b. *Histophilus somni* disease conditions with simultaneous infections by ovine gammaherpesvirus 2 in cattle herds from Southern Brazil. *Braz J Microbiol.* 54: 1159–1169.<https://doi.org/10.1007/s42770-023-00915-5>
- Headley, S. A., Okano, W., Balbo, L. C., Marcasso, R. A., Oliveira, T. E. S., Alfieri, A. F., Negri Filho, L. C., Michelazzo, M. M. Z., Rodrigues, S. M. C., Baptista, A. L., Saut, J. P. E. & Alfieri, A. A. 2018. Molecular survey of infectious agents associated with bovine respiratory disease in a beef cattle feedlot in southern Brazil. *J Vet Diagn Invest.* 30: 249–251.<https://doi.org/10.1177/1040638717739945>
- Hegazy, A. A., Nakai, M., Fuke, N., Hussein, A. E., Kondo, H. & Hirai, T. 2025. Detection of bovine respiratory disease complex-related pathogens in nasopharynx-associated lymphoid tissue. *J Vet Diagn Invest.* 37: 284–297.<https://doi.org/10.1177/10406387251318415>
- IBGE. (2023, 28 September 2025). Pesquisa da Pecuária Municipal [Municipal Livestock Research]. Accessed 1 May 2026, from <https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9107-producao-da-pecuaria-municipal.html?=&t=resultados>.
- Jerez, J. A., Gregori, F., Brandão, P. E., Rodriguez, C. A. R., Ito, F. H., Buzinaro, M. d. G. & Sakai, T. 2005. Isolation of bovine coronavirus (BCoV) in monolayers of HmLu-1 cells. *Braz J Microbiol.* 36: 207–210.<https://doi.org/10.1590/S1517-83822005000300001>
- Kamel, M. S., Davidson, J. L. & Verma, M. S. 2024. Strategies for bovine respiratory disease (BRD) diagnosis and prognosis: a comprehensive overview. *Animals* 14.<https://doi.org/10.3390/ani14040627>
- Lion, A., Secula, A., Rançon, C., Boulesteix, O., Pinard, A., Deslis, A., Hägglund, S., Salem, E., Cassard, H., Näslund, K., Gaudino, M., Moreno, A., Brocchi, E., Delverdier, M., Zohari, S., Baranowski, E., Valarcher, J.-F., Ducatez, M. F. & Meyer, G. 2021. Enhanced pathogenesis caused by Influenza D Virus and *Mycoplasma bovis* coinfection in calves: a disease severity linked with overexpression of IFN-γ; as a key player of the enhanced innate immune response in lungs. *Microbiol Spectr.* 9: e01690–01621.<https://doi.org/10.1128/spectrum.01690-21>

- Madeira, R. B., Fagnani, R., Baptista, A. L., Ferreira, F. P., Reis, M. H., Alves, D. S., Silva, F. H. P., Fenta, M. D., Saut, J. P. E. & Headley, S. A. 2026. Epidemiology, economic losses, and management implications associated with pneumonia-related mortality in feedlots from Brazil. *Pesq Vet Bras.* 46: <https://doi.org/10.1590/1678-5150-PVB-7809>
- Magalhães, L. Q., Baptista, A. L., Fonseca, P. d. A., Menezes, G. L., Nogueira, G. M., Headley, S. A., Fritzen, J. T. T., Alfieri, A. A. & Saut, J. P. E. 2017. Use of metaphylactic protocols based on the risk to develop bovine respiratory diseases in feedlot cattle. *Cienc Rural.* 47: e20161110. <https://doi.org/10.1590/0103-8478cr20161110>
- Mello, J. L., Lorencena, D., Delai, R. R., Kunz, A. F., Possatti, F., Alfieri, A. A. & Takiuchi, E. 2024. A comprehensive molecular analysis of bovine coronavirus strains isolated from Brazil and comparison of a wild-type and cell culture-adapted strain associated with respiratory disease. *Braz J Microbiol.* 55: 1967–1977. <https://doi.org/10.1007/s42770-024-01287-0>
- Mohseni Afshar, Z., Barary, M., Hosseinzadeh, R., Alijanpour, A., Hosseinzadeh, D., Ebrahimpour, S., Nazary, K., Sio, T. T., Sullman, M. J. M., Carson-Chahhoud, K. & Babazadeh, A. 2022. Breakthrough SARS-CoV-2 infections after vaccination: a critical review. *Hum Vaccin Immunother.* 18: 2051412. <https://doi.org/10.1080/21645515.2022.2051412>
- Murray, G. M., More, S. J., Sammin, D., Casey, M. J., McElroy, M. C., O'Neill, R. G., Byrne, W. J., Earley, B., Clegg, T. A., Ball, H., Bell, C. J. & Cassidy, J. P. 2017. Pathogens, patterns of pneumonia, and epidemiologic risk factors associated with respiratory disease in recently weaned cattle in Ireland. *J Vet Diagn Invest.* 29: 20–34. <https://doi.org/10.1177/1040638716674757>
- Ng, T. F., Kondov, N. O., Deng, X., Van Eenennaam, A., Neibergs, H. L. & Delwart, E. 2015. A metagenomics and case-control study to identify viruses associated with bovine respiratory disease. *J Virol.* 89: 5340–5349. <https://doi.org/10.1128/jvi.00064-15>
- Oliveira, T. E. S., Pelaquim, I. F., Flores, E. F., Massi, R. P., Valdiviezo, M. J. J., Pretto, L. G., Alfieri, A. A., Saut, J. P. E. & Headley, S. A. 2020a. *Mycoplasma bovis* and viral agents associated with the development of bovine respiratory disease in adult dairy cows. *Transbound Emerg Dis.* 67: 82–93. <https://doi.org/10.1111/tbed.13223>
- Oliveira, V. H. S., Dall Agnol, A. M., Fritzen, J. T. T., Lorenzetti, E., Alfieri, A. A. & Alfieri, A. F. 2020b. Microbial diversity involved in the etiology of a bovine respiratory disease outbreak in a dairy calf rearing unit. *Comp Immunol Microbiol Infect Dis.* 71: 101494. <https://doi.org/10.1016/j.cimid.2020.101494>
- Ostler, J. B. & Jones, C. 2023. The bovine herpesvirus 1 latency-reactivation cycle, a chronic problem in the cattle industry. *Viruses.* 15. <https://doi.org/10.3390/v15020552>
- Padalino, B., Cirone, F., Zappaterra, M., Tullio, D., Ficco, G., Giustino, A., Ndiana, L. A. & Pratelli, A. 2021. Factors affecting the development of bovine respiratory disease: a cross-sectional study in beef steers shipped from France to Italy. *Front Vet Sci.* 8. <https://doi.org/10.3389/fvets.2021.627894>
- Paller, T., Hostnik, P., Pogacnik, M. & Toplak, I. 2017. The prevalence of ten pathogens detected by a real-time PCR method in nasal swab samples collected from live cattle with respiratory disease. *Slov Vet Res.* 54: 101–107. <https://doi.org/10.26873/SVR-24743-2017>
- Pardon, B. & Buczinski, S. 2020. Bovine respiratory disease diagnosis: what progress has been made in clinical diagnosis? *Vet Clin North Am Food Anim Pract.* 36: 399–423. <https://doi.org/10.1016/j.cvfa.2020.03.004>
- Pedroso, N. H., Dionisio, I. F., Santos, N. R. D., Silva Júnior, J. V. J., Batista, C. P., Decaris, N., Gomes, V., Weiblen, R. & Flores, E. F. 2026. Bacterial and viral agents in cattle with and without respiratory signs in dairy herds from São Paulo and Rio Grande do Sul states, Brazil. *Pesq Vet Bras.* 46: e07742. <https://doi.org/10.1590/1678-5150-PVB-7742>
- Perez-Casal, J. 2020. Pathogenesis and virulence of *Mycoplasma bovis*. *Vet Clin North Am Food Anim Pract.* 36: 269–278. <https://doi.org/10.1016/j.cvfa.2020.02.002>
- Perotta, J. H., Silva, I. V., Rodriguez, M. C., Joineau, M. E. G., Tschá, M. K., Sousa, R. S., Dall Agnol, A. M., Silva, F. H. P., Buczinski, S., Headley, S. A. & Barros Filho, I. R. 2025. Outbreak of respiratory disease due to bovine respiratory syncytial virus with concomitant infections by *Histophilus somni* and *Pasteurella multocida* in adult dairy cows and calves from Southern Brazil. *Animals.* 15. <https://doi.org/10.3390/ani15203015>
- Righi, C., Franzoni, G., Feliziani, F., Jones, C. & Petrini, S. 2023. The cell-mediated immune response against bovine alphaherpesvirus 1 (BoHV-1) infection and vaccination. *Vaccines.* 11: 785. <https://doi.org/10.3390/vaccines11040785>
- Rodriguez, M. C., Silva, I. V., Perotta, J. H., Buczinski, S., Silva, F. H. P., Fenta, M. D., Barros Filho, I. R. & Headley, S. A. 2026. Standardization of a quantitative multiplex real-time PCR system for the detection of bovine respiratory disease pathogens in clinical samples of dairy cattle from southern Brazil. *J Microbiol Methods.* 248: 107639. <https://doi.org/10.1016/j.mimet.2026.107639>
- Saif, L. J. 2010. Bovine respiratory coronavirus. *Veterinary Clinics of North America. Food Animal Practice.* 26: 349–364. <https://doi.org/10.1016/j.cvfa.2010.04.005>
- Santos, Y. G. d., Calheiros, E. B., Santos, E. A., Souza, A. P. A. d., Souza, G. F. C., Macedo, A. T. M., Peixoto, R. d. M., Silva Junior, A. & Costa, M. M. 2025. Prevalence and risk factors of bovine alphaherpesvirus 1 (BoHV-1) in

- western Pernambuco, Brazil. *Revista Brasileira de Saúde e Produção Animal*. 26.<https://doi.org/10.1590/20240025>
- Smith, D. R. 2020. Risk factors for bovine respiratory disease in beef cattle. *Anim Health Res Rev*. 21: 149–152.<https://doi.org/10.1017/S1466252320000110>
- Step, D. L., Krehbiel, C. R., DePra, H. A., Cranston, J. J., Fulton, R. W., Kirkpatrick, J. G., Gill, D. R., Payton, M. E., Montelongo, M. A. & Confer, A. W. 2008. Effects of commingling beef calves from different sources and weaning protocols during a forty-two-day receiving period on performance and bovine respiratory disease. *J Anim Sci*. 86: 3146–3158.<https://doi.org/10.2527/jas.2008-0883>
- Su, Y., Zhao, G., Xu, J. & Wang, Y. 2025. Mapping the global distribution of *Mycoplasma bovis* infections in cattle (2000–2024): A geospatial modeling analysis. *J Dairy Sci*. 108: 10099–10124.<https://doi.org/10.3168/jds.2025-26513>
- Timsit, E., Dendukuri, N., Schiller, I. & Buczinski, S. 2016. Diagnostic accuracy of clinical illness for bovine respiratory disease (BRD) diagnosis in beef cattle placed in feedlots: A systematic literature review and hierarchical Bayesian latent-class meta-analysis. *Prev Vet Med*. 135: 67–73.<https://doi.org/10.1016/j.prevetmed.2016.11.006>
- Timsit, E., Hallewell, J., Booker, C., Tison, N., Amat, S. & Alexander, T. W. 2017. Prevalence and antimicrobial susceptibility of *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* isolated from the lower respiratory tract of healthy feedlot cattle and those diagnosed with bovine respiratory disease. *Vet Microbiol*. 208: 118–125.<https://doi.org/10.1016/j.vetmic.2017.07.013>
- USDA-FAS. (2026). 2025/2026 Beef Production-Top Producing Countries: United States Department of Agriculture-Foreign Agricultural Service. Accessed 14 April 2026, from <https://www.fas.usda.gov/data/production/0111000>.
- Vlasova, A. N. & Saif, L. J. 2021. Bovine coronavirus and the associated diseases. *Front Vet Sci*. Volume 8 - 2021.<https://doi.org/10.3389/fvets.2021.643220>
- Zhang, H., Lu, D., Zhang, Y., Zhao, G., Raheem, A., Chen, Y., Chen, X., Hu, C., Chen, H., Yang, L. & Guo, A. 2022. Annexin A2 regulates *Mycoplasma bovis* adhesion and invasion to embryo bovine lung cells affecting molecular expression essential to inflammatory response. *Front Immunol*. 13.<https://doi.org/10.3389/fimmu.2022.974006>
- Zhang, M., Hill, J. E., Fernando, C., Alexander, T. W., Timsit, E., van der Meer, F. & Huang, Y. 2019. Respiratory viruses identified in western Canadian beef cattle by metagenomic sequencing and their association with bovine respiratory disease. *Transbound Emerg Dis*. 66: 1379–1386.<https://doi.org/10.1111/tbed.13172>
- Zhou, Y., Shao, Z., Dai, G., Li, X., Xiang, Y., Jiang, S., Zhang, Z., Ren, Y., Zhu, Z., Fan, C. & Zhang, G. 2023. Pathogenic infection characteristics and risk factors for bovine respiratory disease complex based on the detection of lung pathogens in dead cattle in Northeast China. *J Dairy Sci*. 106: 589–606.<https://doi.org/10.3168/jds.2022-21929>

Figures

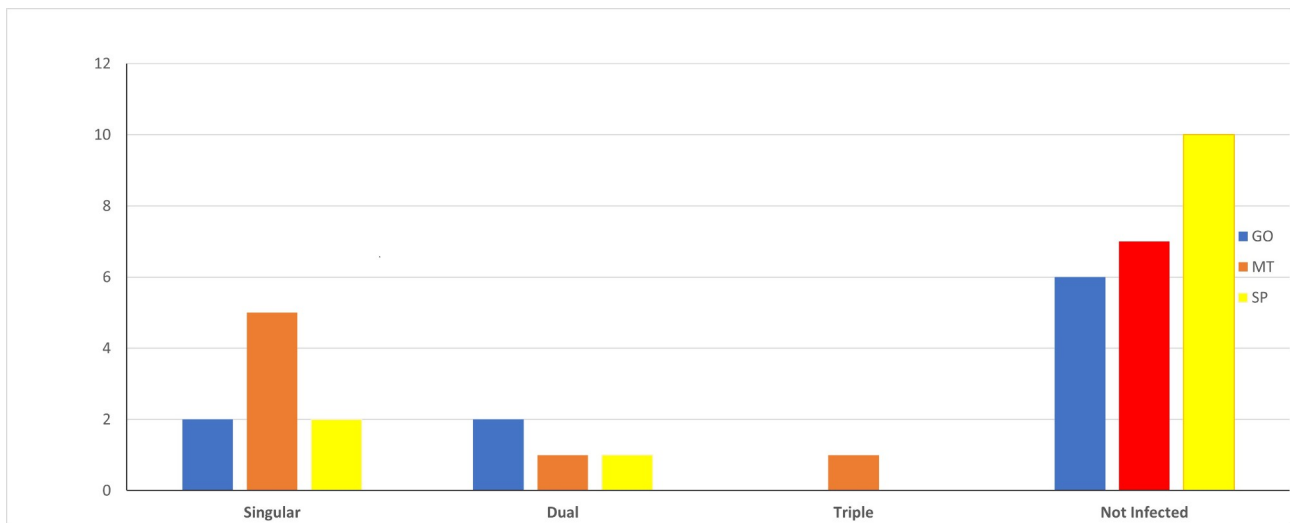


Fig.1. Types of pathogen detection observed in cattle maintained at feedlots from the states of Goiás (GO), São Paulo (SP), and Mato Grosso (MT).

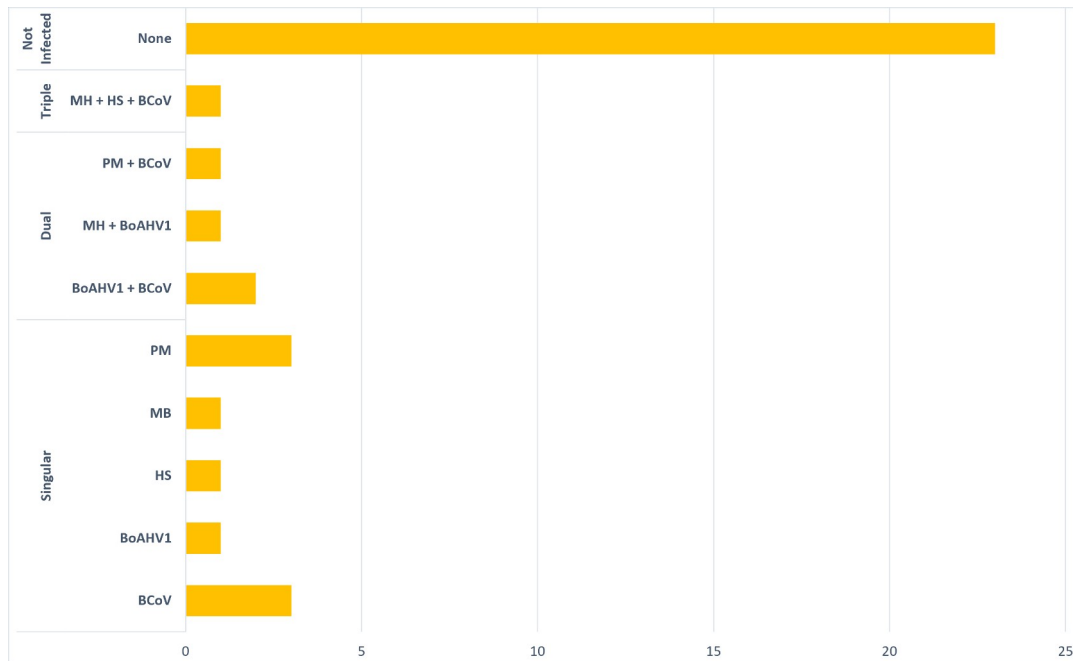


Fig.2. The frequency of pathogen detection observed in steers from three feedlots in Brazil. Legend: MH, *Mannheimia haemolytica*; HS, *Histophilus somni*; PM, *Pasteurella multocida*; MB, *Mycoplasma bovis*; BCoV, bovine coronavirus; BoAHV1, bovine alphaherpesvirus 1.

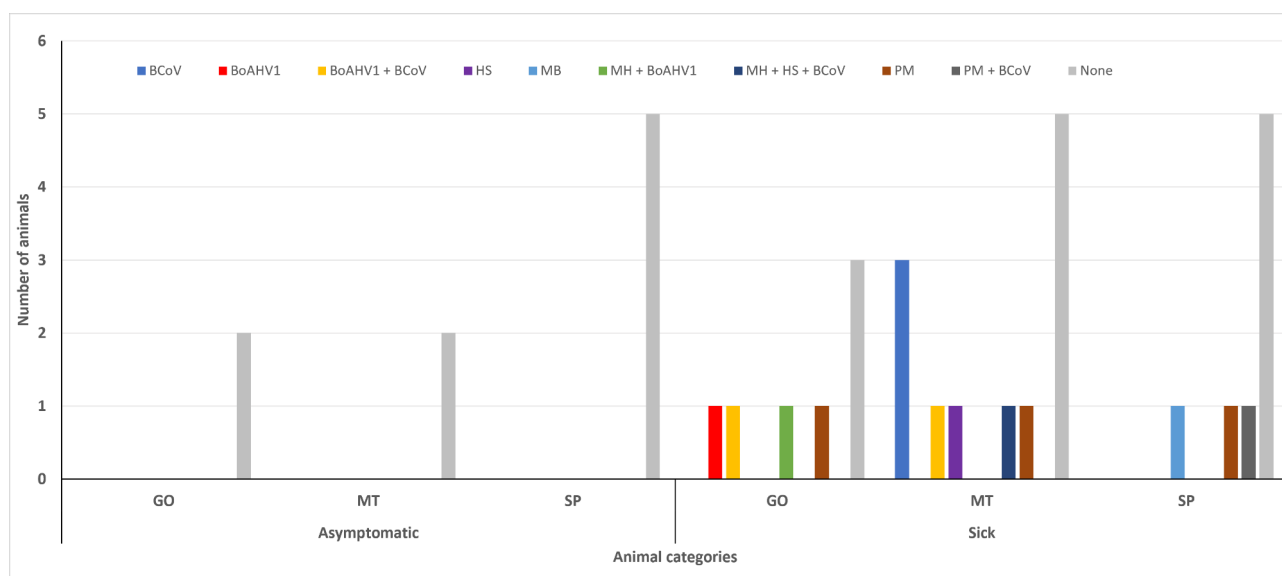


Fig.3. Pathogen detection in asymptomatic and sick steers from three beef cattle feedlots in Brazil. Legend: GO, Goiás; MT, Mato Grosso; SP, São Paulo; MH, *Mannheimia haemolytica*; HS, *Histophilus somni*; PM, *Pasteurella multocida*; MB, *Mycoplasma bovis*; BCoV, bovine coronavirus; BoAHV1, bovine alphaherpesvirus 1.

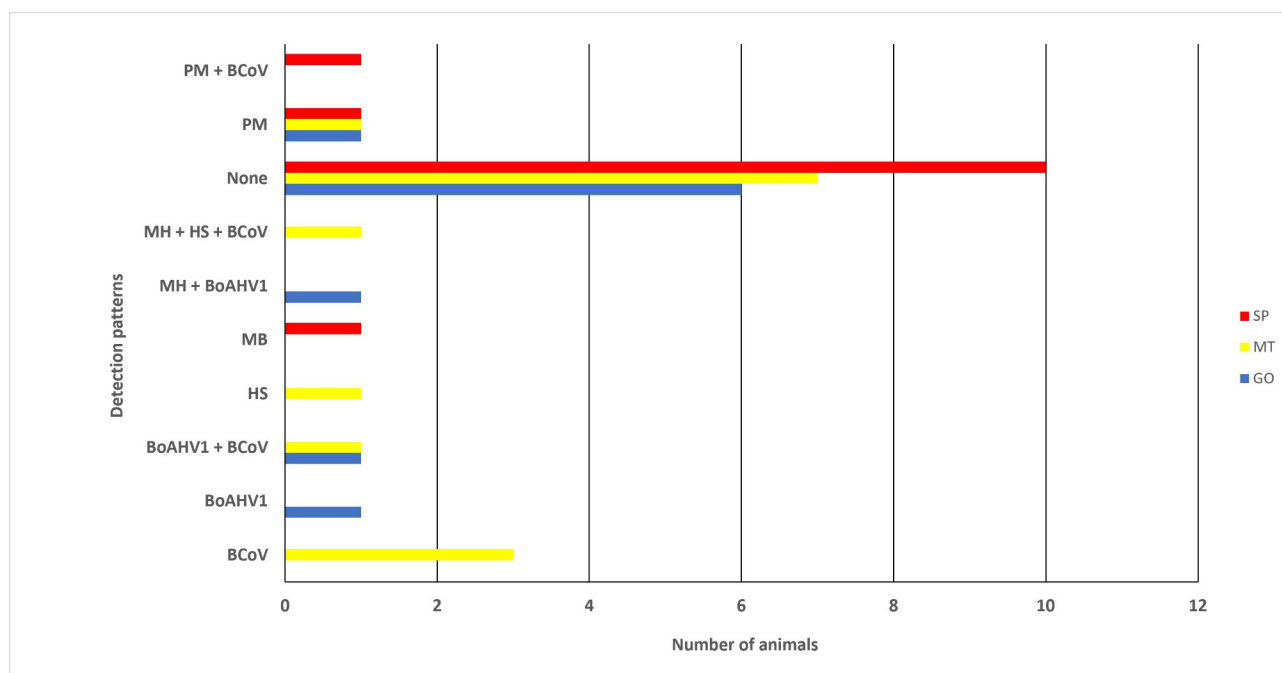


Fig.4. Frequency of single and multiple pathogen detections at each of the three feedlots where cattle were sampled. Legend: GO, Goiás; MT, Mato Grosso; SP, São Paulo; MH, *Mannheimia haemolytica*; HS, *Histophilus somni*; PM, *Pasteurella multocida*; MB, *Mycoplasma bovis*; BCoV, bovine coronavirus; BoAHV1, bovine alphaherpesvirus 1.

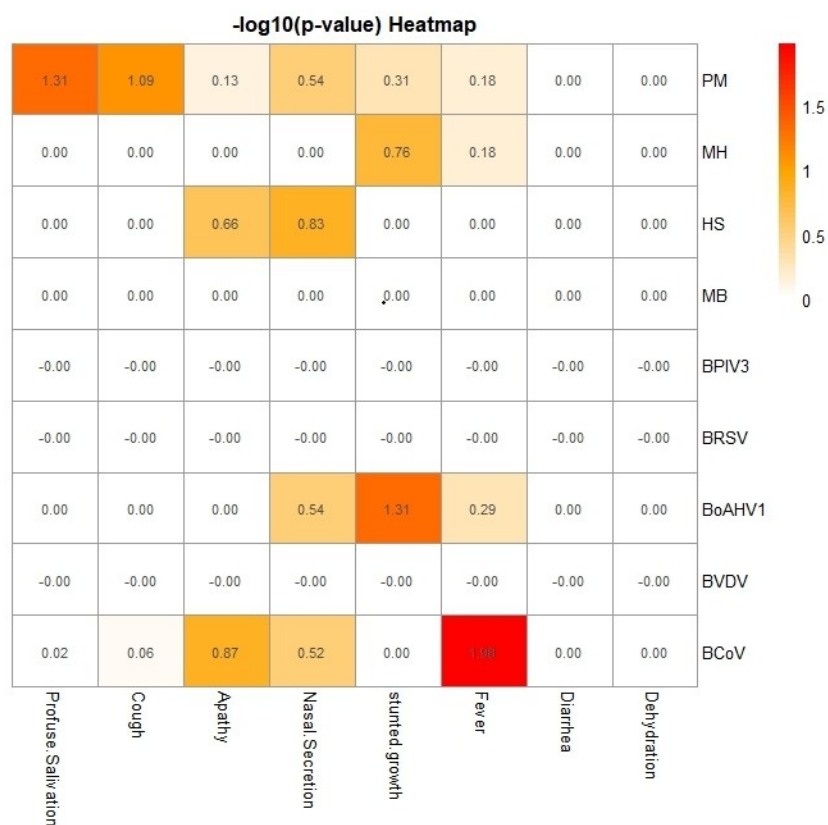


Fig.5. The statistical heat-graph of the association between respiratory pathogen detection and the clinical signs observed in feedlot cattle with respiratory disease. Legend: MH, *Mannheimia haemolytica*; HS, *Histophilus somni*; PM, *Pasteurella multocida*; MB, *Mycoplasma bovis*; BCoV, bovine coronavirus; BVDV, bovine viral diarrhea virus; BoAHV1, bovine alphaherpesvirus 1.

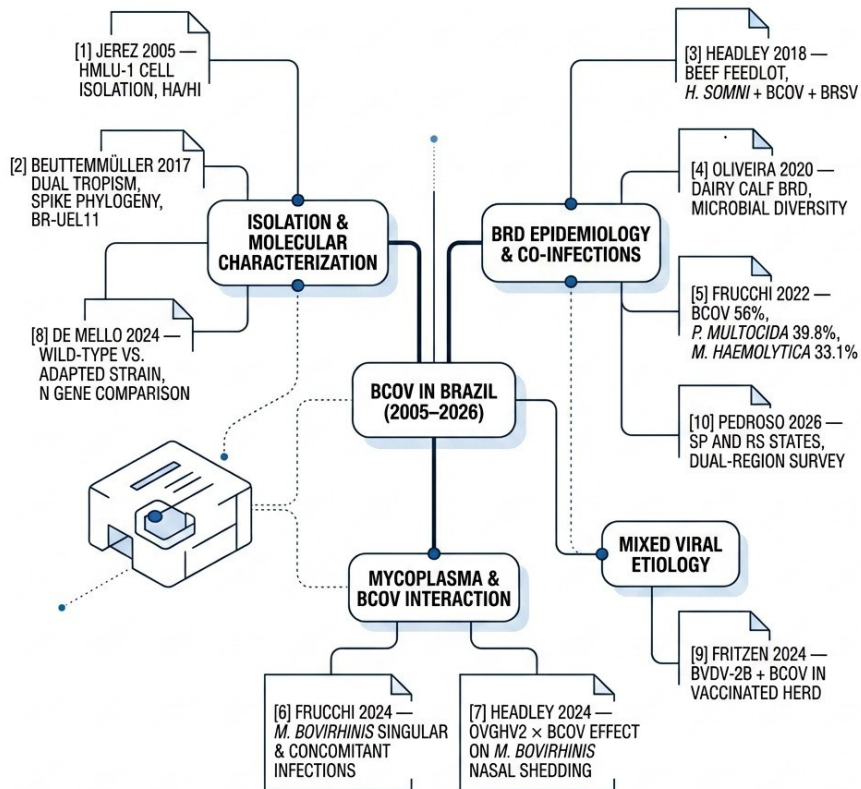


Fig.6. Chronological demonstration of the detection of bovine coronavirus in cattle from Brazil with respiratory disease.

Tables

Table 1. Association between the types of pathogen detection and the geographical locations of the feedlots in Brazil.

Types of detection	Locations of Feedlots			Total ¹
	Goiás ¹	Mato Grosso ¹	São Paulo ¹	
Singular	2	5	2	9
Dual	2	1	1	4
Triple	0	1	0	1
Not detected	6	7	10	23
Total	10	14	13	37

Multivariable analysis					
Type	Coefficient (β)	Std. Error	z-value	p-value	Odds Ratio
Not detected					
Goiás	1.098	0.816	1.345	0.179	3.00
Mato Grosso	0.847	1.345	0.630	0.529	2.33
São Paulo	1.205	1.329	0.906	0.365	3.34
Singular					
Goiás	-0.0003	1.000	-0.0003	0.9998	1.00
Mato Grosso	1.609	1.483	1.085	0.278	5.00
São Paulo	0.694	1.581	0.439	0.661	2.00
Dual					
Goiás	-9.073	66.010	-0.137	0.891	0.00011
Mato Grosso	0.907	66.025	0.137	0.891	2.30
São Paulo	-3.009	425.593	-0.007	0.994	0.049

¹, Number of pathogens detected.

Table 2. Association between the clinical manifestations observed at feedlots and the types of pathogen detection observed in Nelore steers from Brazil.

Variables (present/absent)	Number of animals	Wald Statistic	P-value
Intercept		0.246	0.025
Profuse salivation	5	0.896	0.035
Cough	6	0.091	0.319
Apathy	12	1.299	0.104
Nasal secretion	10	1.170	0.069
Stunted growth	6	0.203	0.012
Fever	13	0.148	0.100
Diarrhea	1	0.032	0.190
Dehydration	3	0.067	0.108

Multinomial Logistic Regression Analysis of Clinical Signs Associated with Singular and Multiple Pathogen Detections				
Types of detection	Variables	Coefficient (β)	P-value	Outcome
Dual	Profuse salivation	-20.34	<0.001	Significant
	Cough	-15.63	<0.001	Significant
	Apathy	19.97	<0.001	Significant
	Nasal secretions	-43.77	<0.001	Significant
	Stunted growth	-58.78	<0.001	Significant
	Fever	-16.12	<0.001	Significant
	Diarrhea	3.26	Not estimated	Sparse data
	Dehydration	-29.15	Not estimated	Sparse data
Singular	Profuse salivation	-19.41	<0.001	Significant
	Cough	34.87	<0.001	Significant
	Apathy	20.22	0.56	Not Significant
	Nasal secretions	-44.75	<0.001	Significant
	Stunted growth	-6.38	<0.001	Significant
	Fever	36.48	<0.001	Significant
	Diarrhea	-3.40	<0.001	Significant
	Dehydration	32.40	<0.001	Significant
Triple	Profuse salivation	-2.83	<0.001	Significant
	Cough	-4.45	<0.001	Significant
	Apathy	1.49	<0.001	Significant
	Nasal secretions	-85.74	<0.001	Significant
	Stunted growth	-25.42	<0.001	Significant
	Fever	37.37	<0.001	Significant
	Diarrhea	0.82	<0.001	Significant
	Dehydration	1.53	<0.001	Significant

Table 3. Demonstration of possible post-vaccination detection of vaccine-included pathogens in feedlot cattle (n=37) immunized at feedlot entry with a commercial respiratory vaccine.

Pathogen contained in commercial vaccine	Potential post-vaccination detection of vaccine-included pathogens	
	Pathogens detected	Prevalence (%)
<i>Pasteurella multocida</i>	3	8.1%
<i>Histophilus somni</i>	2	5.4%
<i>Mannheimia haemolytica</i>	2	5.4%
Bovine alphaherpesvirus 1	2	5.4%