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Livestock Diseases

Bacterial and viral agents in cattle with and without respiratory signs in dairy herds from São Paulo and Rio Grande do Sul states, Brazil[1]

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ABSTRACT.- Pedroso NH, Dionisio IF, dos Santos NRD, Silva Júnior JVJ, Batista CP, Decaris N, Gomes V, Weiblen R, Flores EF. **Bacterial and viral agents in cattle with and without respiratory signs in dairy herds from São Paulo and Rio Grande do Sul states, Brazil.** *Pesquisa Veterinária Brasileira 46:00, 2026.* Setor de Virologia, Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria, Av. Roraima 1000, Prédio 63A, Camobi, Santa Maria, RS 97105-900, Brazil. E-mail: nataliahpedroso@gmail.com

Bovine respiratory disease is a multifactorial syndrome of complex etiology, involving interactions among bacterial and viral agents, and has a significant impact on the cattle industry worldwide. This study aimed to identify agents associated with respiratory infections in dairy herds in São Paulo (SP) and Rio Grande do Sul (RS) states. Nasal swabs obtained from animals with and without nasal/respiratory signs in 10 herds in SP (n = 178) and nine herds in RS (n = 273) were submitted to nucleic acid extraction and to endpoint multiplex reverse transcription polymerase chain reaction (RT-PCR)/PCR assays for bacterial and viral agents. The bacterial multiplex PCR covers four bacteria (*Histophilus somni*, *Mannheimia haemolytica*, *Mycoplasma bovis* and *Pasteurella multocida*); the viral multiplex encompasses seven viruses (bovine herpesvirus 1 – BoAHV-1; bovine viral diarrhea virus 1 and 2 – BVDV-1, BVDV-2; HoBi-like pestivirus – HoBiPeV; bovine respiratory syncytial virus – BRSV; bovine parainfluenza 3 virus – BPIV-3 and bovine coronavirus – BCoV). The overall frequency of positive samples was 23.7% (107/451) and the most frequently detected agents were *H. somni* (30.8%, 33/107) and *M. bovis* (21.5%, 23/107), followed by BCoV (19.6%, 21/107), BPIV-3 (13.1%, 14/107), *M. haemolytica* (11.2%, 12/107) and BRSV (9.3%, 10/107). Mixed infections were detected in 13.1% samples (14/107), especially involving bacteria of the Pasteurellaceae family, but also BCoV and BRSV. Considering herd prevalence, *H. somni* was detected in 57.9% herds (11/19), *M. bovis* in 52.6% (10/19), *M. haemolytica* in 42.1% (8/19), BPIV-3 in 36.8% (7/19), BCoV in 26.3% (5/19), BoAHV-1 and BRSV in 21% (4/19), *P. multocida* in 15.8% (3/19) and BVDV in 5.3% (1/19) herds. Overall, these results offer an overview of the main viral and bacterial agents associated with respiratory infection in dairy herds in the studied regions (SP and RS). In addition, the complex etiology of respiratory disease in cattle highlights the importance of employing multi-etiological diagnostic approaches for comprehensive and accurate investigation.

INDEX TERMS: Bovine respiratory disease, multiplex assay, epidemiology, cattle, viral diseases, bacterial diseases.

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RESUMO.- [**Agentes bacterianos e virais em bovinos com ou sem sinais respiratórios em rebanhos leiteiros dos estados de São Paulo e Rio Grande do Sul, Brasil.**] A doença respiratória bovina é uma síndrome multifatorial de etiologia complexa que envolve interações entre agentes bacterianos e virais, com impacto significativo na indústria pecuária mundial. Este estudo teve como objetivo identificar os agentes relacionados à infecção respiratória em rebanhos leiteiros nos estados de São Paulo (SP) e Rio Grande do Sul (RS). Suabes nasais obtidos de animais com ou sem sinais nasais/respiratórios em dez rebanhos em SP (n = 178) e nove rebanhos no RS (n = 273) foram submetidos à extração de ácidos nucleicos e a testes de transcrição reversa seguida pela reação em cadeia da polimerase (RT-PCR)/PCR *multiplex* convencional para agentes bacterianos e virais. O PCR *multiplex* bacteriano abrange quatro espécies de bactérias (*Histophilus somni*, *Mannheimia haemolytica*, *Mycoplasma bovis* e *Pasteurella multocida*); o *multiplex* viral é capaz de detectar sete vírus (herpesvírus bovino 1 – BoAHV-1; vírus da diarreia viral bovina 1 e 2 – BVDV-1, BVDV-2; pestivírus *HoBi-like* – HoBiPeV; vírus sincicial respiratório bovino – BRSV; vírus da parainfluenza bovina 3 – BPIV-3 e coronavírus bovino – BCoV). A frequência de amostras positivas foi de 23,7% (107/451) e os agentes mais frequentemente detectados foram *H. somni* (30,8%; 33/107) e *M. bovis* (21,5%; 23/107), seguidos por BCoV (19,6%; 21/107), BPIV-3 (13,1%; 14/107), *M. haemolytica* (11,2%; 12/107) e BRSV (9,3%; 10/107). Infecções mistas foram detectadas em 13,1% das amostras (14/107), especialmente envolvendo bactérias da família Pasteurellaceae, mas também BCoV e BRSV. Considerando a prevalência de rebanhos, *H. somni* foi detectado em 57,9% dos rebanhos (11/19), *M. bovis* em 52,6% (10/19), *M. haemolytica* em 42,1% (8/19), BPIV-3 em 36,8% (7/19), BCoV em 26,3% (5/19), BoAHV-1 e BRSV em 21% (4/19), *P. multocida* em 15,8% (3/19) e BVDV em 5,3% (1/19) dos rebanhos. No geral, esses resultados fornecem uma visão geral dos principais agentes virais e bacterianos associados com infecção respiratória em rebanhos leiteiros nas regiões estudadas (SP e RS). Além disso, a etiologia complexa das doenças respiratórias em bovinos justifica o uso abordagens diagnósticas multietiológicas, capazes de fornecer um diagnóstico mais abrangente e preciso.

TERMOS DE INDEXAÇÃO: Doença respiratória bovina, ensaio *multiplex,* epidemiologia, gado, doenças virais, doenças bacterianas.

INTRODUCTION

Bovine respiratory disease (BRD) is a respiratory illness of cattle with a complex multifactorial etiology, usually involving bacterial and viral agents (Chai et al. 2022, Kamel et al. 2024). The disease is distributed worldwide and has an important sanitary and economic impact on dairy and beef cattle operations, mainly in feedlots and herds with high animal density (Smith 2020).

The main BRD agents include the bacteria *Histophilus somni, Mannheimia haemolytica*, *Mycoplasma bovis* and *Pasteurella multocida*, and viruses *Varicellovirus bovinealpha1* (bovine herpesvirus 1 – BoAHV-1), bovine pestiviruses (Pestivirus bovis, bovine viral diarrhea virus – BVDV-1; *Pestivirus tauri*, BVDV-2 and Pestivirus brazilense, HoBi-like pestivirus – HoBiPeV); *Respirovirus bovis 3* (bovine parainfluenza virus 3 – BPIV-3), *Orthopneumovirus bovis* (bovine respiratory syncytial virus – BRSV) and *Betacoronavirus gravedinis* (bovine coronavirus – BCoV) (Smith et al. 2020, Castro et al. 2021, Goto et al. 2023, ICTV 2024). The potential multiagent etiology hampers the understanding of BRD pathogenesis, its etiological diagnosis and the establishment of adequate control measures (Kamel et al. 2024, Zhang et al. 2020).

Several studies have described the occurrence of BRD in Brazil, particularly in the southern region. Oliveira et al. (2020b) identified a wide range of microbial diversity in a dairy calf rearing unit in southern Brazil, with the most frequently detected pathogens being *P. multocida*, *H. somni* and *M. bovis*, along with viruses, such as BRSV and BCoV. Studies conducted in Paraná state have highlighted *H. somni* and *M. bovis*, both being commonly identified in young and adult dairy cattle (Massi et al. 2023, Alfieri et al. 2024). Similarly, Headley et al. (2018) reported high detection rates of *H. somni*, BRSV, BCoV and *M. haemolytica* in cattle from the same region. Consistent findings were also reported by Oliveira et al. (2020a), who observed high detection rates of *M. bovis*, BVDV, BoAHV-1, BRSV and BPIV-3 in adult dairy cows in Paraná. Supporting these molecular findings, serological data from unvaccinated cows in Paraná indicated a high prevalence of BoAHV-1, BVDV, BPIV-3 and BRSV (Yoshitani et al. 2024).

Despite its evident sanitary and economic relevance in Brazil (Assis Brasil et al. 2013, Baptista et al. 2017), epidemiological data on BRD remain limited, as most reports are confined to outbreak investigations and provide only partial information on potential pathogens (Flores et al. 2000, Assis Brasil et al. 2013, Beuttemmuller et al. 2017, Oliveira et al. 2021). This scenario results in a limited understanding of the current circulation and importance of some respiratory agents in Brazilian cattle.

Herein, we conducted a multiplex reverse transcription polymerase chain reaction (RT-PCR)/PCR-based investigation of bacterial and viral agents in animals with and without nasal/respiratory signs suggestive of BRD in dairy herds from São Paulo (SP) and Rio Grande do Sul (RS) states, Brazil.

MATERIALS AND METHODS

Ethical approval. The study did not involve animal experimentation. The samples used in the study were obtained from the diagnostic service offered by the "Setor de Virologia" (Virology Section – SV) of "Universidade Federal de Santa Maria" (UFSM) and collaborating institutions ("Universidade de São Paulo" – protocol no. 5689310119).

Nasal secretions were collected from animals with or without nasal/respiratory signs belonging to 10 dairy herds in SP (2020–2021) and nine in RS (2025). Nasal swabs were submitted to the SV-UFSM for processing as described below.

São Paulo (SP) herds. Swab samples (n = 178) collected from female calves in SP state during 2020–2021 (Decaris et al. 2022) included animals with and without clinical signs of BRD. The study by Decaris et al. (2022) was approved by the Animal Care and Use Committee of the School of Veterinary Medicine and Animal Science, University of São Paulo (protocol no. 5689310119). Sample collection was conducted from July 2020 to January 2021, covering the winter, spring and summer seasons, on 10 dairy farms (Fig. 1). The study included pre-weaning Holstein heifer calves aged between 30 and 127 days (median of 59 days) that had not been vaccinated against BRD in the 15 days prior to sampling (Decaris et al. 2022). Among the farms evaluated, two did not perform BRD vaccination as part of their regular practices.

Rio Grande do Sul (RS) herds. Swab samples (n = 273) were collected by veterinarians in nine mid-sized to large dairy herds in central and northwestern RS, regions that harbor important dairy basins (Fig. 2). The collection period spanned from late March to mid-May 2025, during which the average temperature ranged from 3.4 °C to 32.9 °C. Monthly precipitation averaged 93 mm (INMET 2025). Samples were collected from calves, heifers and cows presenting respiratory signs (nasal secretion and/or cough) during the daily inspection and stored at -20 °C until shipment to the laboratory for testing. These herds usually vaccinate for the main agents of respiratory disease using commercial multivalent vaccines. According to veterinarian information, none of the animals have been treated with antibiotics or other antimicrobial drugs before swab collection. The samples were submitted to SV-UFSM as part of a collaborative effort to identify the main respiratory agents circulating in these herds.

Swab processing and nucleic acid extraction. Nasal swabs immersed in 1 mL of PBS were stored at -80 °C until processing. The tubes were centrifuged (1,190 × *g* for 10 min), and 100 µL of the supernatant was used for nucleic acid extraction. DNA/RNA extraction was performed using TRIzol (Invitrogen™)-based protocol (Pedroso et al. 2023), and nucleic acid was eluted in 40 µL of Tris-EDTA (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The concentration and quality of the extracted nucleic acids were assessed using a NanoDrop Lite Spectrophotometer (Thermo Scientific).

Multiplex PCR/RT-PCR assays. The extracted nucleic acids were submitted in parallel to a viral multiplex PCR/RT-PCR assay and to a bacterial multiplex PCR assay (Pedroso 2025). Briefly, samples were screened for BoAHV-1, bovine pestiviruses (BVDV-1, BVDV-2 and HoBiPeV), BPIV-3, BRSV and BCoV; and *Histophilus somni*, *Mannheimia haemolytica*, *Mycoplasma bovis* and *Pasteurella multocida*. PCR/RT-PCR products were resolved in 5% agarose gels stained with GelRed.

Statistical analysis. The difference in positivity between the states was analyzed using the chi-square test (95% confidence interval, $p < 0.05$).

RESULTS

Nucleic acids (DNA/RNA) extracted from nasal secretions collected from animals with/without nasal/respiratory signs were submitted to bacterial and viral multiplex PCR/RT-PCR assays. The bacterial multiplex covers four bacteria, and the viral assay encompasses seven viral species frequently associated with respiratory disease in cattle.

The overall positive sample frequency was 23.7% (107/451). The most frequently detected agents were *Histophilus somni* (30.8%, 33/107) and *Mycoplasma bovis* (21.5%, 23/107), followed by BCoV (19.6%, 21/107), BPIV-3 (13.1%, 14/107), *Mannheimia haemolytica* (11.2%, 12/107) and BRSV (9.3%, 10/107) (Fig. 3). Co-detections were detected in 13.1% samples (14/107), especially involving bacteria of the Pasteurellaceae family, but also BCoV and BRSV (Fig. 4). Considering herd prevalence, *H. somni* was detected in 57.9% herds (11/19), *M. bovis* in 52.6% (10/19), *M. haemolytica* in 42.1% (8/19), BPIV-3 in 36.8% (7/19), BCoV in 26.3% (5/19), BoAHV-1 and BRSV in 21.1% (4/19), *Pasteurella multocida* in 15.8% (3/19) and BVDV in 5.3% (1/19) herds (Table 1).

The frequency of positive samples in SP farms was 24.7% (44/178) for at least one of the agents (Fig. 5). Among viral targets, 10 samples were positive for BCoV (22.7%, 10/44), six for BPIV-3 (13.6%, 6/44), four for BRSV (9.1%, 4/44), three for BoAHV-1 (6.8%, 3/44) and one for pestiviruses

Fig. 1. Geographic distribution of sampled dairy herds in São Paulo, Brazil. The dots indicate the cities in the counties where the herds were sampled.

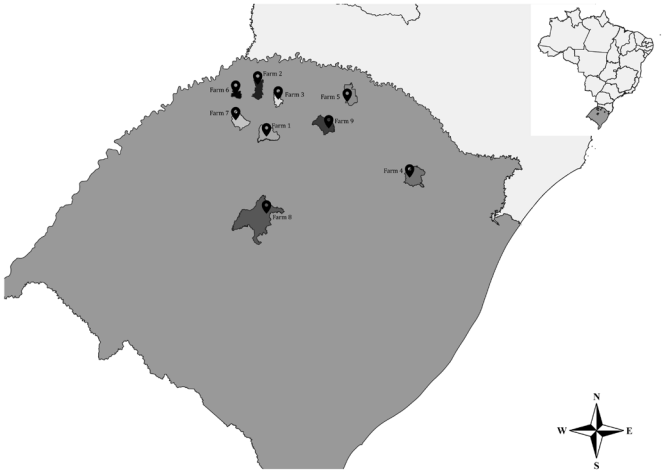

Fig. 2. Geographic distribution of sampled dairy herds in Rio Grande do Sul, Brazil. The dots indicate the cities in the counties where the herds were sampled.

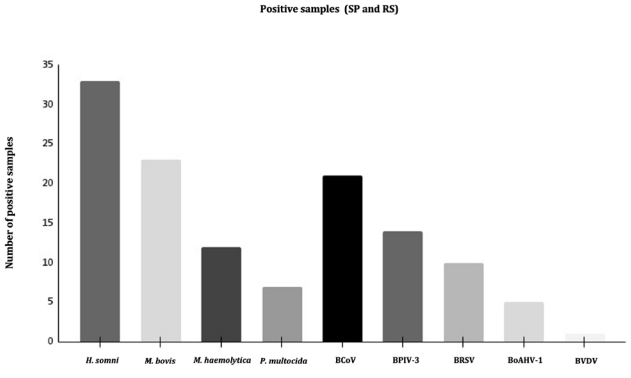

Fig. 3. Positive samples for bacterial and viral agents in nasal secretions of cattle with or without nasal/respiratory signs from dairy herds in São Paulo (SP) and Rio Grande do Sul (RS), Brazil.

(2.3%, 1/44). Regarding bacterial agents, 12 samples were positive for *M. bovis* (27.3%; 12/44), six for *H. somni* (13.6%; 6/44), and five for *M. haemolytica* (11.4%; 5/44). No samples tested positive for *P. multocida*. Co-detections were identified in three samples (6.8%, 3/44): BCoV and *H. somni*; BCoV and *M. bovis*; BRSV and *M. haemolytica*.

Among RS farms, a total of 63 swabs (23.1%, 63/273) tested positive for at least one agent (Fig. 6). Of these, 52 (82.5%, 52/63) had single infections and were distributed across nine farms in RS. The frequency of single viral infections was: 15.4% BPIV-3 (8/52), 13.5% BCoV (7/52), 11.5% BRSV (6/52), 3.8% BoAHV-1 (2/52). Pestiviruses were not detected in any sample. The most frequent single bacterial infection was *H. somni* (30.8%, 16/52), followed by *M. bovis* (21.2%, 11/52) and *M. haemolytica* (3.8%, 2/52). Co-detections were identified particularly among bacterial agents of the Pasteurellaceae family (17.5%, 11/63). Calves represented the majority of positive samples, comprising 60.3% (38/63) of detections. Co-detections were common in this group of positive samples (90.9%, 10/11), whereas a single co-detection (BCoV and *H. somni*) was found in cows. No statistically significant differences were observed between the states ($p > 0.05$).

DISCUSSION

This article describes the molecular detection of bacterial and viral agents in nasal secretions of cattle with and without clinical signs in dairy herds in SP (2020–2021) and RS (2025). The results provide an overview of the main respiratory pathogens circulating in these dairy herds and, in extension, in herds of the respective regions. In addition, our results reinforce the need to include multiple agents in the laboratory diagnosis of respiratory disease in cattle, especially through the use of multiplex molecular assays.

A high frequency of *Histophilus somni* and *Mycoplasma bovis* was detected in nasal swabs from cattle in both states. The abundance of these bacteria in the bovine nasal microbiome is a predictor of the development of clinical signs of BRD (Chai et al. 2022, Centeno-Delphia et al. 2025). In Brazil, *H. somni* has already been identified in association with respiratory disease manifestations in the southern regions (Headley et al. 2017, 2018, Oliveira et al. 2020b) and appears to have a neglected role in dairy herds, given its recent emergence with high prevalence in adult animals (Massi et al. 2023). This pattern was also observed in this study, in which *H. somni* was detected not only in adult cows but also in calves, highlighting its epidemiological significance across different animal categories.

Another agent that has been somewhat neglected is *M. bovis*, whose role in the pathogenesis of BRD has long been underestimated due to difficulties in its laboratory identification (Dudek & Nicholas 2024). However, it is now widely recognized as a primary pathogen involved in cases linked to increased mortality and fatal pneumonia in cattle in Brazil (Oliveira et al. 2020a, Massi et al. 2023, Alfieri et al. 2024) and in other countries as well (Fanelli et al. 2021, Sedó et al. 2025).

Conversely, *Pasteurella multocida* and *Mannheimia haemolytica* were seldomly detected, in agreement with previous reports

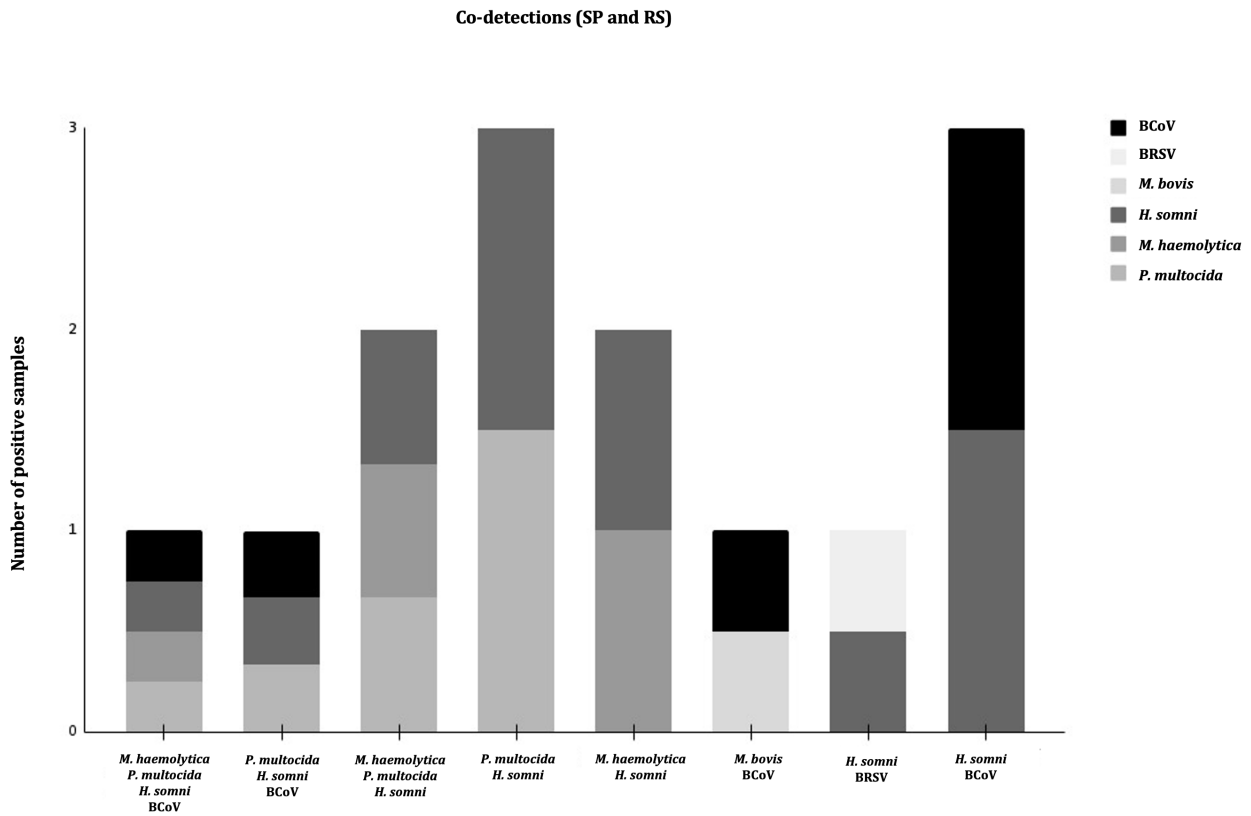

Fig. 4. Co-detections of bacteria and viral agents in nasal swabs from dairy herds from São Paulo (SP) and Rio Grande do Sul (RS), Brazil.

from dairy herds in southern Brazil (Frucchi et al. 2022, Massi et al. 2023). When present, these bacteria are often associated with co-detections, highlighting their opportunistic nature (Massi et al. 2023, Alfieri et al. 2024). Despite inconclusive evidence regarding the low detection rates of *P. multocida* and *M. haemolytica* (Larson & Step 2012), routine vaccination in

Table 1. Herd-level distribution of positive samples and detected agents in dairy farms from São Paulo and Rio Grande do Sul, Brazil

Herd	County	Lactating cows (n)	Total samples	Agents (n) (including co-detections)
Farm 1	Condor (RS)	1010	30	*Mycoplasma bovis* (3) *Histophilus somni* (1)
Farm 2	Redentora (RS)	263	30	BRSV (5) *H. somni* (3) *M. bovis* (2) BCoV (1) *Mannheimia haemolytica* (1)
Farm 3	Boa Vista das Missões (RS)	131	30	BPIV-3 (2) BoAHV-1 (1)
Farm 4	Ipê (RS)	203	30	*H. somni* (4) *M. haemolytica* (1)
Farm 5	Erechim (RS)	103	32	*H. somni* (3) *Pasteurella multocida* (2) *M. haemolytica* (1) BPIV-3 (1)
Farm 6	Sede Nova (RS)	105	30	*H. somni* (5) *M. haemolytica* (2) BPIV-3 (2)
Farm 7	Chiapetta (RS)	287	31	*M. bovis* (3) *H. somni* (2) BCoV (1) BPIV-3 (1) BRSV (1)
Farm 8	Julio de Castilhos (RS)	1150	30	*H. somni* (4) *M. bovis* (2) *P. multocida* (1) BCoV (1) BoAHV-1 (1) BPIV-3 (1)
Farm 9	Pontão (RS)	235	30	*H. somni* (5) *P. multocida* (4) *M. haemolytica* (2) *M. bovis* (1) BPIV-3 (1) BCoV (8)
Farm 10	Bragança Paulista (SP)	170	11	-
Farm 11	Piracicaba (SP)	314	21	*M. bovis* (4)
Farm 12	Bernardino de Campos (SP)	590	28	*M. bovis* (2)
Farm 13	Araras (SP)	2076	18	*M. haemolytica* (3) *M. bovis* (4)
Farm 14	Águas da Prata (SP)	590	21	*H. somni* (2) *M. bovis* (1)
Farm 15	Santa Rita do Passa Quatro (SP)	85	5	BRSV (2) *M. haemolytica* (1)
Farm 16	Caçapava (SP)	118	15	BRSV (2) *H. somni* (2) *M. haemolytica* (1)
Farm 17	Itapetininga (SP)	170	21	BoAHV-1 (1)
Farm 18	São Pedro (SP)	292	28	BCoV (10) *H. somni* (2) *M. bovis* (1)
Farm 19	Santa Cruz da Conceição (SP)	120	10	BPIV-3 (6) BoAHV-1 (2) BVDV (1)

BRSV = bovine respiratory syncytial virus, BcoV = bovine coronavirus, BPIV-3 = bovine parainfluenza 3 virus, BoAHV-1 = bovine herpesvirus 1, BVDV = bovine viral diarrhea virus.

these herds may have contributed to the low prevalence of these pathogens observed in the present study.

Among viruses, BCoV was the most frequent pathogen detected in samples from both states, both in single and co-detections, followed by BPIV-3 and BRSV. The high prevalence of BCoV has been observed in studies conducted in beef and dairy cattle in Brazil (Beuttemmuller et al. 2017, Oliveira et al. 2020b, Frucchi et al. 2022), as well as in North America and Europe (Pratelli et al. 2021, Denholm et al. 2024, Sedó et al. 2025). Similarly, the relevance of BPIV-3 circulation in Brazilian cattle herds is evidenced by our findings and agreement with previous serological studies and viral isolation (Sardi et al. 2002, Gonçalves et al. 2003, Gaeta et al. 2017, Yoshitani et al. 2024), while BRSV is frequently reported as a common viral agent in cattle exhibiting clinical signs in RS and SP

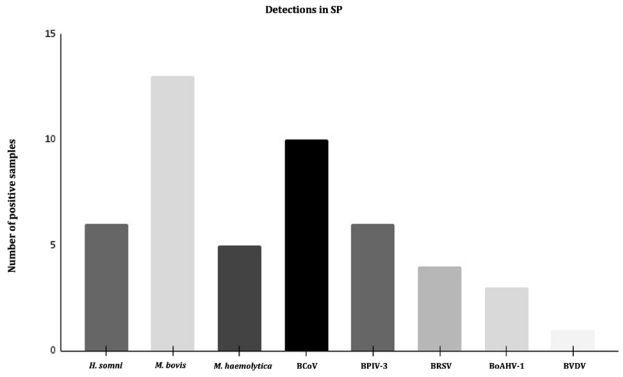

Fig. 5. Detection of bacterial and viral agents in nasal secretions from cattle presenting or not presenting nasal/respiratory signs, sampled from dairy herds located in São Paulo (SP), Brazil. Co-detections were identified in three samples from SP (bovine coronavirus – BCoV and *Histophilus somni*; BCoV and *Mycoplasma bovis*; bovine respiratory syncytial virus – BRSV and *Mannheimia haemolytica*).

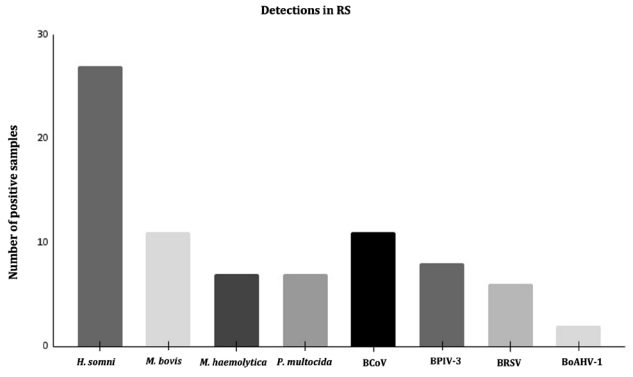

Fig. 6. Detection of bacterial and viral agents in nasal secretions from cattle with nasal/respiratory signs from dairy herds from Rio Grande do Sul (RS), Brazil. Co-detections were identified in 11 samples from RS: bovine coronavirus (BCoV) + *Histophilus somni* + *Pasteurella multocida* + *Mannheimia haemolytica* (1/11); BCoV + *H. somni* + *P. multocida* (1/11); *H. somni* + *P. multocida* + *M. haemolytica* (2/11); BCoV + *H. somni* (2/11); *M. haemolytica* + *H. somni* (2/11) and *H. somni* + *P. multocida* (3/11).

(Driemeier et al. 1997, Flores et al. 2000, Domingues et al. 2011, Assis Brasil et al. 2013, Oliveira et al. 2020b). The low frequency of BoAHV-1 and pestiviruses may be attributed, in part, to vaccine-induced protection, in line with other studies reporting similar low rates (Frucchi et al. 2022, Bernal et al. 2023, Massi et al. 2023, Buczinski et al. 2024). In this sense, most sampled herds (17/19) reported systematic vaccination with respiratory vaccines, which include BoAHV-1 and BVDV.

Sample collection in SP state was conducted from July 2020 to January 2021 on 10 dairy farms. It included pre-weaning Holstein heifer calves (mean age: 59 days), regardless of the presence of clinical respiratory signs. Among the samples, 24.7% (44/178) tested positive for at least one respiratory pathogen. The most frequently detected agents were *M. bovis* (27.3%) and BCoV (22.7%), and co-detections of viral and bacterial pathogens were observed in 6.8% of the positive samples. These coinfections are relatively common in BRD. The viral-bacterial superinfection model is the most studied in BRD, with BRSV, BVDV, BoHV-1, and BPIV-3 enhancing bacterial colonization and clinical severity (Gaudino et al. 2022). Viral-viral and bacterial-bacterial coinfections also occur, but remain poorly studied (Gaudino et al. 2022).

In RS, the samples were collected from calves, heifers and cows in mid- to large-sized herds in free-stall and compost barn operations during April and May, 2025. Collections were performed from each animal category upon initial nasal and/or respiratory signs (serous or mucosal nasal discharge and/or ocular discharge). No follow-up was performed in these animals after sample collection. In this sampling, 23.1% of the samples (63/273) tested positive for at least one agent, predominantly in single infections (82.5%), with *H. somni* (30.8%), *M. bovis* (21.2%), BPIV-3 (15.4%) and BCoV (13.5%) being the most common; calves represented the majority of positive cases (60.3%). Laboratory detection in asymptomatic animals or those with early respiratory signs is essential to avoid underestimating disease prevalence, which can occur when diagnosis relies solely on clinical evaluation.

The main limitations of this study include the transversal/sectional nature of the collection and the lack of follow-up monitoring of the affected animals. Considering the different sampling periods in SP (2020–2021) and RS (2025), variations in pathogen detection would be expected, thereby limiting direct comparisons between the two datasets. However, despite these temporal and regional discrepancies, the main detected targets were overall similar in both samplings. It should be noted that, in RS, the winter season, during which the frequency of respiratory disease increases, had not yet begun by the time sample collection ceased (Bernal et al. 2023). In addition, all herds had a history of vaccination with respiratory vaccines, which could have influenced the detection rates of the pathogens investigated (Bernal et al. 2023, Buczinski et al. 2024), except for two farms located in SP. Importantly, the limited number of herds included in this study (n = 19) precludes an extrapolation of the results to the respective dairy basins (SP and RS). Thus, the results may be considered an overview of the main agents circulating in the sampled herds at the respective periods of specimen collection rather than a broad figure of agents circulating in the respective states.

Another limitation of the study is the absence of emergent agents in our investigation, *e.g., Deltainfluenzavirus influenzae*

(bovine influenzavirus D – IDV), *Macavirus ovinegamma2* (ovine gammaherpesvirus 2 – OvGHV2) and *Mastadenovirus bostertium* (bovine adenovirus 3 – BAdV-3). These agents have also been occasionally associated with respiratory signs in several countries, and at least some of them may also be circulating in Brazilian herds (Silva et al. 2022, Goto et al. 2023, Headley et al. 2023).

CONCLUSION

Overall, the results presented herein provide an overview of the main respiratory agents circulating in dairy herds in these regions at the time of sample collection. In addition, these results indicate the need to include several agents in the etiological diagnosis of respiratory disease in cattle, preferably through the use of multiplex tests.

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REFERENCES

Alfieri AF, Fritzen JTT, Yasumitsu CY, Alfieri AA. Microbiological profile of the upper and lower respiratory tract of suckling and weaned dairy calves with acute respiratory disease. Vet Sci 2024; https://doi.org/10.3390/vetsci11100493

Assis Brasil ND, Hinnah FL, Fiss L, Sallis ESV, Grecco FB, Ladeira SRL, Marcolongo-Pereira C, Schild AL. Doenças respiratórias em bezerros na região sul do Rio Grande do Sul: estudo retrospectivo de 33 surtos. Pesq Vet Bras 2013; https://doi.org/10.1590/S0100-736X2013000600010

Baptista AL, Rezende AL, Fonseca PA, Massi RP, Nogueira GM, Magalhães LQ, Headley SA, Menezes GL, Alfieri AA, Saut JPE. Bovine respiratory disease complex associated mortality and morbidity rates in feedlot cattle from southeastern Brazil. J Infect Dev Ctries 2017; https://doi.org/10.3855/jidc.9296

Bernal JMC, Fernández A, Arnal JL, Baselga C, Zuñiga AB, Fernández-Garayzábal JF, Alonso AIV, Cid D. Cluster analysis of bovine respiratory disease (BRD)-associated pathogens shows the existence of two epidemiological patterns in BRD outbreaks. Vet Microbiol 2023; https://doi.org/10.1016/j.vetmic.2023.109701

Beuttemmuller EA, Alfieri AF, Headley SA, Alfieri AA. Brazilian strain of bovine respiratory coronavirus is derived from dual enteric and respiratory tropism. Genet Mol Res 2017; https://shre.ink/qQus

Buczinski S, Broes A, Savard C. Frequency of bovine respiratory disease complex bacterial and viral agents using multiplex real-time qPCR in Quebec, Canada, from 2019 to 2023. Vet Sci 2024; https://doi.org/10.3390/vetsci11120631

Castro MM, Oliveira TES, Headley SA. Bovine respiratory disease in Brasil: a short review. Semina Ciênc Agrár 2021; https://doi.org/10.5433/1679-0359.2021v42n3Supl1p2081

Centeno-Delphia RE, Glidden N, Long E, Ellis A, Hoffman S, Mosier K, Ulloa N, Cheng JJ, Davidson JL, Mohan S, Kamel M, Szasz JI, Schoonmaker J, Koziol J, Boerman JP, Ault A, Verma MS, Johnson TA. Nasal pathobiont abundance is a moderate feedlot-dependent indicator of bovine respiratory disease in beef cattle. Anim Microbiome 2025; https://doi.org/10.1186/s42523-025-00387-y

Chai J, Capik SF, Kegley B, Richeson JT, Powell JG, Zhao J. Bovine respiratory microbiota of feedlot cattle and its association with disease. Vet Res 2022; https://doi.org/10.1186/s13567-021-01020-x

Decaris N, Buczinski S, Tárdon DIC, Camargo L, Schllemer NR, Hagen SCF, Woolums AR, Gomes V. Diagnostic accuracy of Wisconsin and California scoring systems to detect bovine respiratory disease in preweaning dairy calves under subtropical environmental conditions. J Dairy Sci 2022; https://doi.org/10.3168/jds.2021-21491

Denholm K, Evans NP, Baxter-Smith K, Burr P. Retrospective study of the relative frequency of cattle respiratory disease pathogens in clinical laboratory samples submitted by UK veterinary practices. Vet Rec 2024; https://doi.org/10.1002/vetr.4434

Domingues HG, Spilki FR, Arns CW. Detecção molecular e análise filogenética de vírus respiratório sincicial bovino (BRSV) em swabs e tecido pulmonar de bovinos adultos. Pesq Vet Bras 2011; https://doi.org/10.1590/S0100-736X2011001100004

Driemeier D, Gomes MJP, Moojen V, Arns CW, Vogg G, Kessler L, Costa UM. Manifestação clínico-patológica de infecção natural pelo Vírus Respiratório Sincicial Bovino (BRSV) em bovinos de criação extensiva no Rio Grande do Sul, Brasil. Pesq Vet Bras 1997; https://doi.org/10.1590/S0100-736X1997000200006

Dudek K, Nicholas RAJ. Recent role of microorganisms of the Mollicutes class in the etiology of bovine respiratory disease. Pathogens 2024; https://doi.org/10.3390/pathogens13110951

Fanelli A, Cirilli M, Lucente MS, Zarea AAK, Buonavoglia D, Tempesta M, Greco G. Fatal calf pneumonia outbreaks in Italian dairy herds involving *Mycoplasma bovis* and other agents of BRD complex. Front Vet Sci 2021; https://doi.org/10.3389/fvets.2021.742785

Flores EF, Weiblen R, Medeiros M, Botton SA, Irigoyen LF, Driemeier D, Schuch LF, Moraes MS. A retrospective search for bovine respiratory syncytial virus (BRSV) antigens in histological specimens by immunofluorescence and immunohistochemistry. Pesq Vet Bras 2000; https://doi.org/10.1590/S0100-736X2000000400002

Frucchi APS, Agnol AMD, Bronkhorst DE, Beuttemmuller EA, Alfieri AA, Alfieri AF. Bovine coronavirus co-infection and molecular characterization in dairy calves with or without clinical respiratory disease. Front Vet Sci 2022; https://doi.org/10.3389/fvets.2022.895492

Gaeta NC, Ribeiro BLM, Alemán MAR, Thomazelli LM, Durigon EL, Nogueira AHC, Stefano E, Okuda LH, Pituco EM, Gregory L. Evaluation of bovine parainfluenza type-3 virus and influenza virus D participation in bovine respiratory disease of calves from Brazilian family farming. Med Vet UFRPE 2017; https://shre.ink/AyCE

Gaudino M, Nagamine B, Ducatez MF, Meyer G. Understanding the mechanisms of viral and bacterial coinfections in bovine respiratory disease: a comprehensive literature review of experimental evidence. Vet Res 2022; https://doi.org/10.1186/s13567-022-01086-1

Gonçalves DA, Spilki FR, Chiminazzo C, Oliveira MA, Franco AC, Roehe PM. Isolamento do vírus Parainfluenza bovino tipo 3 no Rio Grande do Sul, Brasil. Ciência Rural 2003; https://doi.org/10.1590/S0103-84782003000500025

Goto Y, Fukunari K, Suzuki T. Multiplex RT-qPCR application in early detection of bovine respiratory disease in healthy calves. Viruses 2023; https://doi.org/10.3390/v15030669

Headley SA, Agnol AMD, Bessegato JA, Frucchi APS, Maturana EFL, Rodrigues RV, Xavier AAC, Alfieri AF, Alfieri AA. Association of ovine gammaherpesvirus 2 with an outbreak of acute respiratory disease in dairy cattle. Sci Rep 2023; https://doi.org/10.1038/s41598-023-30133-w

Headley SA, Balbo LC, Alfieri AF, Saut JPE, Baptista AL, Alfieri AA. Bovine respiratory disease associated with *Histophilus somni* and bovine respiratory syncytial virus in a beef cattle feedlot from Southeastern Brazil. Semina Ciênc Agrár 2017; https://doi.org/10.5433/1679-0359.2017v38n1p283

Headley SA, Okano W, Balbo LC, Marcasso RA, Oliveira TE, Alfieri AF, Negri Filho LC, Michelazzo MZ, Rodrigues SC, Baptista AL, Saut JPE, Alfieri AA. Molecular survey of infectious agents associated with bovine respiratory disease in a beef cattle feedlot in southern Brazil. J Vet Diagn Invest 2018; https://doi.org/10.1177/1040638717739945

ICTV. Taxonomy Browser. Virus Taxonomy: 2024 release. International Committee on Taxonomy of Viruses; 2024. Accessed on May 13, 2025. https://ictv.global/taxonomy

INMET. Banco de Dados Meteorológicos para Ensino e Pesquisa (BDMEP). Instituto Nacional de Meteorologia, Brasília, DF; 2025. Accessed on May 13, 2025. https://bdmep.inmet.gov.br/

Kamel MS, Davidson JL, Verma MS. Strategies for bovine respiratory disease (BRD) diagnosis and prognosis: a comprehensive overview. Animals 2024; https://doi.org/10.3390/ani14040627

Larson RL, Step DL. Evidence-based effectiveness of vaccination against *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in feedlot cattle for mitigating the incidence and effect of bovine respiratory disease complex. Vet Clin N Am Food Anim Pract 2012; https://doi.org/10.1016/j.cvfa.2011.12.005

Massi RP, Lunardi M, Alfieri AF, Alfieri AA. Neglected bacterial infections associated to bovine respiratory disease in lactating cows from high-yielding dairy cattle herds. Braz J Microbiol 2023; https://doi.org/10.1007/s42770-023-01165-1

Oliveira TES, Pelaquim IF, Flores EF, Massi RP, Valdiviezo MJJ, Pretto-Giordano LG, Alfieri AA, Saut JPE, Headley SA. *Mycoplasma bovis* and viral agents associated with the development of bovine respiratory disease in adult dairy cows. Transb Emerg Dis 2020a; https://doi.org/10.1111/tbed.13223

Oliveira TES, Scuisato GS, Pelaquim IF, Cunha CW, Cunha LS, Flores EF, Pretto-Giordano LG, Lisbôa JAN, Alfieri AA, Saut JPE, Cunha PHG, Headley SA. The participation of a malignant catarrhal fever virus and *Mycoplasma bovis* in the development of single and mixed infections in beef and dairy cattle with bovine respiratory disease. Front Vet Sci 2021; https://doi.org/10.3389/fvets.2021.691448

Oliveira VHS, Agnol AMD, Fritzen JTT, Lorenzetti E, Alfieri AA, Alfieri AF. Microbial diversity involved in the etiology of a bovine respiratory disease outbreak in a dairy calf rearing unit. Comp Immunol Microbiol Infect Dis 2020b; https://doi.org/10.1016/j.cimid.2020.101494

Pedroso NH, Silva Júnior JVJ, Becker AS, Weiblen R, Flores EF. An end-point multiplex PCR/reverse transcription-PCR for detection of five agents of bovine neonatal diarrhea. J Microbiol Methods 2023; https://doi.org/10.1016/j.mimet.2023.106738

Pedroso NH. Desenvolvimento e aplicação de testes PCR/RT-PCR multiplex para detecção de agentes da doença respiratória bovina. Tese de Doutorado, Universidade Federal de Santa Maria, Santa Maria, 2025. 94p.

Pratelli A, Cirone F, Capozza P, Trotta A, Corrente M, Balestrieri A, Buonavoglia C. Bovine respiratory disease in beef calves supported long transport stress: An epidemiological study and strategies for control and prevention. Res Vet Sci 2021; https://doi.org/10.1016/j.rvsc.2020.11.002

Sardi SI, Campos GS, Barros SB, Edelweiss GL, Martins DT. Detecção de anticorpos contra o vírus da parainfluenza bovina tipo 3 (PI-3) e o vírus da leucose bovina (VLB) em bovinos de diferentes municípios do Estado da Bahia, Brasil. Rev Ci Méd Biol 2002; https://repositorio.ufba.br/bitstream/ri/1532/1/3211.pdf

Sedó SGU, Winder CB, Perry KV, Caswell JL, Mee JF, MacNicol J, Renaud DL. Herd-level occurrence and risk factors associated with respiratory and enteric pathogens from dairy calves in Ontario: a cross-sectional study. J Dairy Sci 2025; https://doi.org/10.3168/jds.2024-25199

Silva MS, Mosena ACS, Baumbach L, Demoliner M, Gularte JS, Pavarini SP, Driemeier D, Weber MN, Spilki FR, Canal CW. Cattle influenza D virus in Brazil is divergent from established lineages. Arch Virol 2022; https://doi.org/10.1007/s00705-022-05416-8

Smith DR. Risk factors for bovine respiratory disease in beef cattle. Anim Health Res Rev 2020; https://doi.org/10.1017/S1466252320000110

Smith RA, Step DL, Woolums AR. Bovine respiratory disease: looking back and looking forward, what do we see? Vet Clin N Am Food Anim Pract 2020; https://doi.org/10.1016/j.cvfa.2020.03.009

Yoshitani GD, Camilo SLO, Fritzen JTT, Oliveira MV, Lorenzetti E, Lisbôa JAN, Alfieri AF, Alfieri AA. Serological profile for major respiratory viruses in unvaccinated cows from high-yielding dairy herds. Animals 2024; https://doi.org/10.3390/ani14091256

Zhang M, Hill JE, Godson DL, Ngeleka M, Fernando C, Huang Y. The pulmonary virome, bacteriological and histopathological findings in bovine respiratory disease from western Canada. Transbound Emerg Dis 2020; https://doi.org/10.1111/tbed.13419