Evaluation of Mollicutes Microorganisms in Respiratory Disease of Cattle and Their Relationship to Clinical Signs

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Background: Bovine respiratory disease (BRD) is an important problem in cattle production that is responsible for economic losses in dairy herds. Mycoplasma spp. are described as an important etiological agent of BRD.

Hypothesis: To evaluate the occurrence of the most important mycoplasmas in the lower respiratory tract of healthy and BRD cattle in relationship to clinical signs of BRD.

Methods: Tracheal lavage samples were collected and added to tubes containing Hayflick media. Mycoplasma spp. were identified by the presence of “fried egg” like colonies, biochemical tests and polymerase chain reaction (PCR). Occurrence of Mollicutes, M. bovis, M. mycoides subsp. mycoides SC and M. dispar was evaluated. The association between clinical signs of BRD and the presence of Mycoplasma spp. also was evaluated.

Results: Colonies were obtained from a 1-year-old BRD calf only. However, species identification was not possible. Mollicutes (P = .035) and M. dispar (P = .036) were more common in BRD cattle. The relationship between Mollicutes and crackle (P = .057) was not significant. M. dispar was associated to tachypnea (P = .045) and mixed dyspnea (P = .003). Relationships to heart rate (P = .062) and crackle (P = .062) were not significant.

Conclusions and clinical importance: The results confirmed the importance of mycoplasma as an etiologic agent of BRD and suggested M. dispar as part of the respiratory microbiota and its possible role in the development of BRD.

Key words: Bovine; Boviatrics; Diseases of cattle; Mycoplasma.

BRD bovine respiratory disease
MmmSC Mycoplasma mycoides subsp. mycoides small colony
PCR polymerase chain reaction
mycoides small colony is the etiological agent of contagious bovine pleuropneumonia, and it is considered the most pathogenic mycoplasma. Mycoplasma mycoides subsp. mycoides SC has never been detected in Brazilian cattle, although its detection in the external auditory meatus of clinically healthy goats was described elsewhere. M. bovis is an opportunistic bacterium considered part of the bovine respiratory tract microbiota. After stressful situations, M. bovis becomes pathogenic and clinical signs of BRD are observed, especially in young calves. M. dispar was first isolated from pulmonary lungs of cattle, and it has been described as a potential pathogen associated with BRD.

Considering the importance of Mycoplasma spp. in the development of BRD, the aim of our study was to evaluate the occurrence of the most important mycoplasma species in the lower respiratory tract of healthy and sick Brazilian cattle in relationship to clinical signs of BRD.

Materials and Methods
The study was conducted at the Internal Medicine Department, School of Veterinary Medicine and Animal Science, University of São Paulo and at the Laboratory of Mycoplasmas, Institute of Biomedical Sciences, University of São Paulo, Brazil. All procedures were carried out in agreement with the guidelines of Ethical Principles in Animal Research adopted by the Ethic Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science of University of São Paulo.

Sixty young dairy cattle were randomly selected and enrolled in the study. Fifty-eight cattle were from 10 farms located in the state.
of São Paulo, Brazil. Calves were immediately separated from their mothers after birth. They received colostrum and milk by farm employees, and after weaning, they received a pasture and barley-based diet and mineral salt. Two cattle were presented at the Veterinary Hospital of the School of Veterinary Medicine and Animal Science, University of São Paulo.

**Case Definition**

Bovine respiratory disease was diagnosed when the animal showed ≥2 of the following clinical signs: mucopurulent or purulent nasal discharge, cough, rectal temperature ≥39.5°C, respiratory rate >40 breaths/min, and increased cranioventral lung sounds or crackle. The limits of the lung field were 12° intercostal space at iliac line and 11° intercostal space at sciatric line. Two experienced veterinarians on our research team performed the physical examinations in all cattle. Animals were allocated in 2 groups: healthy (n = 28) and BRD cattle (n = 32).

**Sample Collection**

The distal part of the neck was shaved and decontaminated with 70% alcohol and iodopovidone. Twenty milliliters of sterile saline 0.9% was instilled with a 16 × 40 mm needle and up to 5 mL was recovered. Samples were added to tubes containing Hayflick media and transported on ice to the laboratory.

**Cultivation of Mycoplasma spp.**

Clinical samples were diluted (10⁰, 10⁻¹, 10⁻², 10⁻³) in phosphate-buffer saline (PBS). *Mycoplasma* spp. isolation was performed by plating 100 µL of each dilution in Hayflick media growth plates and adding 200 µL of each dilution in 1800 mL of liquid media containing Hayflick media. Plates and liquid media were incubated at 37°C for 21 days and evaluated on a daily basis. Plates containing “fried-egg” colonies and glucose fermentation with or without arginine hydrolysis were considered positive. Liquid media containing glucose fermentation with or without arginine hydrolysis were considered positive. Liquid media containing glucose fermentation with or without arginine hydrolysis and absence of turbidity were considered positive.

**Molecular Detection**

Molecular investigation of *Mycoplasma* spp was performed using DNA extraction according to a previously described procedure. Polymerase chain reaction was performed to investigate the presence of *Mollicutes* class bacteria. Positive samples were used to detect *M. bovis*, *M. dispar*, and *MmmSC*. Specific PCR tests to detect *M. dispar*, *M. bovis*, and *MmmSC* were performed in 25% (8 of 32) and 64% (18 of 28) of samples from healthy and BRD groups, respectively, because of the low quality of the other samples. *M. dispar* was increased in BRD cattle (61%) compared to the healthy group (12.5%; *P* = .036). *M. bovis* was detected in BRD animals only (5%), and no difference between groups was noted (*P* = .497).

**Statistical Methods**

Descriptive analysis was performed to determine absolute and relative frequencies. The occurrence of *Mycoplasma* spp. was considered the dependent variable. Health status and clinical signs were considered the independent variables. The association between the presence of *Mycoplasma* spp. and health status and clinical signs of BRD was compared by applying the Pearson’s chi-square test or Fisher’s exact test using a 95% confidence interval. Clinical data were analyzed by the Statistical Package for Social Sciences 19.0. Variables with *P* < .05 were considered significant.

**Results**

Clinical signs detected during physical examination of cattle are described in Table 1. Most healthy cattle showed only normal findings. However, some cattle showed lethargy (6%), expiratory or inspiratory dyspnea (3%), mixed dyspnea (both expiratory and inspiratory dyspnea) (3%), crackle (3%), or snoring (3%).

Colonies were obtained from 1 BRD calf only. However, species identification was not possible because of the low quality of the sample.

Polymerase chain reaction was performed to detect *Mollicutes*, *M. dispar*, *M. bovis*, and *MmmSC*. *Mollicutes* were increased in BRD cattle (68%) compared to healthy cattle (41%; *P* = .035; Table 2). Specific PCR tests to detect *M. dispar*, *M. bovis*, and *MmmSC* were performed in 25% (8 of 32) and 64% (18 of 28) of samples from healthy and BRD groups, respectively.

**Table 1.** Clinical signs detected after clinical examination of healthy and BRD cattle.

| Clinical Signs | Healthy % (N/T) | BRD % (N/T) | Total % (N/T) |
|---------------|----------------|-------------|---------------|
| Behavior      |                |             |               |
| Alert         | 94 (30/32)     | 39 (11/28)  | 72 (41/60)    |
| Lethargic     | 06 (02/32)     | 68 (17/28)  | 28 (19/60)    |
| Ocular mucous membrane |   |             |               |
| Normal        | 100 (32/32)    | 75 (21/28)  | 88 (53/60)    |
| Pale          | –              | 25 (07/28)  | 12 (07/60)    |
| Heart rate    |                |             |               |
| <100 bpm      | 100 (32/32)    | 21 (06/28)  | 63 (38/60)    |
| >100 bpm      | –              | 79 (22/28)  | 37 (22/60)    |
| Respiratory rate |            |             |               |
| <40 breaths/min | 100 (32/32) | 36 (10/28)  | 70 (42/60)    |
| >40 breaths/min | –             | 64 (18/28)  | 30 (18/60)    |
| Body temperature |            |             |               |
| <39.5°C       | 100 (32/32)    | 50 (14/28)  | 77 (46/60)    |
| >39.5°C       | –              | 50 (14/28)  | 23 (14/60)    |
| Nasal discharge |              |             |               |
| Absent        | 97 (31/32)     | 18 (05/28)  | 60 (36/60)    |
| Serous        | –              | 11 (03/28)  | 25 (03/60)    |
| Mucous        | 03 (01/32)     | 50 (14/28)  | 25 (15/60)    |
| Mucopurulent/purate | – | 21 (06/28)  | 10 (06/60)    |
| Cough         |                |             |               |
| Absent        | 100 (32/32)    | 18 (05/28)  | 62 (37/60)    |
| Productive    | –              | 100 (15/28) | 25 (15/60)    |
| Nonproductive | –              | 100 (08/28) | 13 (08/60)    |
| Dyspnea       |                |             |               |
| Absent        | 94 (30/32)     | 21 (06/28)  | 60 (36/60)    |
| Inspiratory   | 03 (01/32)     | 11 (03/28)  | 07 (04/60)    |
| Expiratory    | –              | 25 (07/28)  | 12 (07/32)    |
| Mixed         | 03 (01/32)     | 43 (12/28)  | 22 (13/60)    |
| Crackles      |                |             |               |
| Absent        | 97 (31/32)     | 11 (03/28)  | 57 (34/60)    |
| Present       | 03 (01/32)     | 71 (20/28)  | 35 (21/60)    |
| Snoring       |                |             |               |
| Absent        | 97 (31/32)     | 43 (12/28)  | 72 (43/60)    |
| Present       | 03 (01/32)     | 57 (16/28)  | 28 (17/60)    |
| Whistling     |                |             |               |
| Absent        | 100 (32/32)    | 73 (20/28)  | 87 (52/60)    |
| Present       | –              | 27 (08/28)  | 13 (08/60)    |

*Inspiratory and expiratory dyspnea.*
Table 2. Mollicutes, M. dispar, and M. bovis associated to bovine respiratory disease in the state of São Paulo, Brazil.

| Microorganism | Healthy % (N/T) | BRD % (N/T) | OR (CI 95%) | P-value |
|---------------|----------------|-------------|-------------|---------|
| Mollicutes    | 41 (13/32)     | 68 (19/28)  | 3.085 (1.067–8.919) | .035    |
| M. dispar     | 12.5 (01/08)   | 61 (11/18)  | 11.00 (1.103–109.674) | .036    |
| M. bovis      | 00 (00/08)     | 06 (01/18)  | –           | .497    |

Table 2). Mycoplasma mycoides subsp. mycoides SC was not detected. Undetermined species were observed in both healthy (87.5%; 07/08) and BRD groups (33%; 06/18).

The association between the bacteria detected and clinical signs of BRD was evaluated. (Table 3). With regard to M. dispar, tachypnea was more common in positive animals (66.7%) as compared to negative animals (21.4%; \( P = .045 \)). Mixed dyspnea (inspiratory and expiratory dyspnea) was more common in the positive group (66.7%) compared to the negative group (7%; \( P = .003 \)). No significant association between clinical signs and M. dispar was observed (Table 4).

Discussion

To better understand the importance of Mycoplasma spp. in BRD, we evaluated the occurrence of Mycoplasma bovis, Mycoplasma dispar, and Mycoplasma mycoides subsp. mycoides SC in tracheal wash samples of healthy and BRD cattle in association with clinical signs of BRD. Our results indicated that M. dispar was common in BRD animals, confirming its importance as a pathogen of BRD. Association between Mollicutes and some clinical signs of respiratory diseases was detected.

Colonies were obtained from 1 sample only, unlike the high isolation rates of Mycoplasma spp. described elsewhere.5,15,25,26 Mycoplasma spp. are well-known as fastidious and slow-growing bacteria for which isolation takes an extended time.27 Polymerase chain reaction is a quick and sensitive test that can detect nucleic acid from only 1 microorganism when it is used to detect Mollicutes.21 Often, culture negative samples are positive for molecular detection, as observed in our study.

Mollicutes was increased in the BRD group (\( P = .035 \)). Similarly, Mollicutes have been reported frequently in more BRD calves (90.96%; 53%) compared to healthy calves (52.05%; 23%).27,28 Mollicutes are well characterized as part of the bovine respiratory tract microbiota27,29 but several species have been described as etiologic agents of respiratory diseases.30

M. dispar was increased in BRD cattle (\( P = .036 \)). Our data are in agreement with a previous study,31 which also described the increased occurrence of M. dispar in both healthy and BRD groups, especially in the latter group. Two other studies also detected a high occurrence of M. dispar in BRD cattle compared to healthy cattle.12,27 Mycoplasma mycoides subsp. mycoides SC is the etiologic agent of contagious bovine pleuropneumonia, and it is considered the most important mycoplasma species related to BRD.10 In our study, this species was not detected, and this result is in accordance with other Brazilian studies.27,31 M. bovis is another important mycoplasma related to BRD.29,32 In
our study, however, this bacterium was detected in 1 BRD calf only. Similar results were obtained in another study.\textsuperscript{33} Undetermined mycoplasma species were observed in both groups. \textit{Ureaplasma diversum}, \textit{Acholeplasma} spp., and other mycoplasma species such as \textit{M. bovirhinis}, \textit{M. alkalensis}, and \textit{M. arginini} have been detected in the bovine respiratory tract.\textsuperscript{15,16,25–27} The genus \textit{Mycoplasma} has several species, and culture-independent techniques are indispensable to determine all species present in the respiratory tract.

Regarding clinical signs of BRD, our data identified \textit{Mollicutes} and \textit{M. dispar} associated with respiratory problems. Our results establish association but not necessarily causation. Another study found that the presence of a clinical sign of BRD (stony dull sound on percussion of the thorax) was related to the absence of \textit{Mollicutes.}\textsuperscript{31} Regarding \textit{M. dispar}, our data indicated an association between this bacterium and tachypnea and mixed dyspnea. In an experimental infection with \textit{M. dispar} in calves, most calves showed no clinical signs of BRD.\textsuperscript{34} However, only 1 calf showed persistent

Table 4. \textit{M. dispar} associated with clinical signs of bovine respiratory disease in the state of São Paulo, Brazil.

| Clinical Sign                   | Absent (%) | Present (%) | OR         | P-value |
|--------------------------------|------------|-------------|------------|---------|
| Behavior                       |            |             |            |         |
| Alert                          | 64.3       | 41.7        | 2.520 (0.516–12.296) | .249    |
| Lethargic                      | 35.7       | 58.3        |            |         |
| Mucosas                        |            |             |            |         |
| Pink                           | 78.6       | 75          | 1.222 (0.197–7.594) | 1.000   |
| Pale                           | 21.4       | 25          |            |         |
| Hear Rate                      |            |             |            |         |
| <100 bpm                       | 64.3       | 25          | 5.400 (0.983–29.668) | .062    |
| >100 bpm                       | 35.7       | 75          |            |         |
| Respiratory Rate               |            |             |            |         |
| <40 breaths/min                | 78.6       | 33.3        | 7.333 (1.272–42.294) | .045    |
| >40 breaths/min                | 21.4       | 66.7        |            |         |
| Rectal Temperature             |            |             |            |         |
| <39.5°C                        | 78.6       | 50          | 3.667 (0.666–20.191) | .218    |
| >39.5°C                        | 21.4       | 150         |            |         |
| Purulent Nasal discharge       |            |             |            |         |
| Absence                        | 93         | 75          | 4.333 (0.386–48.610) | .306    |
| Presence                       | 07         | 25          |            |         |
| Serous nasal discharge         |            |             |            |         |
| Absence                        | 85.7       | 100         | –          | .483    |
| Presence                       | 14.3       | 0           |            |         |
| Mucous nasal discharge         |            |             |            |         |
| Absence                        | 71.4       | 58.3        | 1.786 (0.349–9.127) | .683    |
| Presence                       | 28.6       | 41.7        |            |         |
| Productive cough               |            |             |            |         |
| Absence                        | 85.7       | 50          | 6.000 (0.919–39.185) | .090    |
| Presence                       | 14.3       | 50          |            |         |
| Nonproductive cough            |            |             |            |         |
| Absence                        | 78.6       | 75          | 1.222 (0.197–7.594) | 1.000   |
| Presence                       | 21.4       | 25          |            |         |
| Mixed dyspnea                  |            |             |            |         |
| Absence                        | 93         | 33.3        | 26.000 (2.451–275.826) | .003    |
| Presence                       | 07         | 66.7        |            |         |
| Expiratory dyspnea             |            |             |            |         |
| Absence                        | 85.7       | 91.7        | 0.545 (0.043–6.889) | 1.000   |
| Presence                       | 14.3       | 08.3        |            | .483    |
| Inspiratory dyspnea            |            |             |            |         |
| Absence                        | 85.7       | 100         | –          | .483    |
| Presence                       | 14.3       | 00          |            |         |
| Crackles                       |            |             |            |         |
| Absence                        | 64.3       | 25          | 5.400 (0.983–29.668) | .062    |
| Presence                       | 35.7       | 75          |            | .462    |
| Snoring                        |            |             |            |         |
| Absence                        | 65.3       | 50          | 1.800 (0.373–8.681) | .462    |
| Presence                       | 35.7       | 50          |            |         |
| Whistling                      |            |             |            |         |
| Absence                        | 78.6       | 75          | 1.222 (0.197–7.594) | 1.000   |
| Presence                       | 21.4       | 25          |            |         |
nonproductive cough and dyspnea, besides increased respiratory rate and fever. Recently, another study indicated that coarse crackles and whistling were associated with the absence of \textit{M. dispar}.\textsuperscript{31} \textit{Mycoplasma dispar} is regularly isolated from bovine pneumonia lungs, but its presence has been associated with mild infection.\textsuperscript{30,34} Discrepancies in results allow researchers to continue studying these microorganisms to better understand the importance of mycoplasmas in the development of clinical signs of BRD. In addition, it is important to note that other microorganisms could contribute to BRD.

**Conclusion**

Our study confirmed the importance of mycoplasmas as etiologic agents of BRD. Although \textit{M. dispar} has been detected in healthy cattle, the increased occurrence of this bacterium and the detection of \textit{M. bovis} in BRD calves confirm their roles in the pathogenesis of BRD.

The increased frequency of undetermined mycoplasma species in samples indicates the complexity of the respiratory tract microbiome and the possible role of other mycoplasmas in BRD. This new information about the association between some clinical signs of BRD and \textit{Mycoplasma} spp. infection will be useful in the presumptive identification of the microorganisms involved in BRD infection.

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**Conflict of Interest Declaration:** Authors declare no conflict of interest

**Off-label Antimicrobial declaration:** Authors declare no off-label use of antimicrobials

**References**

1. Edwards TA. Control methods for bovine respiratory disease for feedlot cattle. Vet Clin North Am Food Anim Pract [Internet] 2010;26:273–284.
2. USDA. Feedlot 2001. Part IV: Health and Health Management on U.S. Feedlots with a capacity of 1,000 or more head. In: APHIS. Fort Collins: National Animal Health Monitoring System; 2013.
3. Hilton WM. BRD in 2014: Where have we been, where are we now, and where do we want to go? Anim Health Res Rev 2014;15:120–122.
4. Hartel H, Nikunen S, Neuvonen E, et al. Viral and bacterial pathogens in bovine respiratory disease in Finland. Acta Vet Scand 2004;45:193–200.
5. Virtal A, Mechor GD, Gröhn YT, Erb HN. Morbidity from nonrespiratory diseases and mortality in dairy heifers during the first three months of life. J Am Vet Med Assoc 1996;208:2043–2046.
6. Lima SF, Teixeira AGV, Higgins CH, et al. The upper respiratory tract microbiome and its potential role in bovine respiratory disease and otitis media. Sci Rep 2016;1:29050.
7. Cernicihario N, White BJ, Renter DG, Babcock AH. Evaluation of economic and performance outcomes associated with the number of treatments after an initial diagnosis of bovine respiratory disease in commercial feeder cattle. Am J Vet Res 2013;74:300–309.
8. Griffin D, Chengappa MM, Kuszak J, McVey DS. Bacterial pathogens of the bovine respiratory disease complex. Vet Clin North Am Food Anim Pract 2010;26:381–294.
9. Griffin D. Bovine pasteurellosis and other bacterial infections of the respiratory tract. Vet Clin North Am - Food Animal Pract 2010;26:57–71.
10. OIE. Contagious Bovine Pleuropneumonia. In: Terrestrial Manual Online. Organização Internacional de Epizootias; 2014; 1–16.
11. Pereira LO, Danelli M, das GM, et al. Identificação molecular de Mycoplasma mycoides subesp. mycoides tipo SC isolado do conduto auditivo externo de caprinos clinicamente sãos. Ciência Rural 2003;33:367–368.
12. Ter Laak EA, Noordergraaf JH, Boomsluiter E. The nasal mycoplasmal flora of healthy calves and cows. J Vet Med 1992;39:610–616.
13. Gabinaitiene A, Siugzdaitė J, Zilinskas H, Siugzdė RPS. Mycoplasma bovis and bacterial pathogens in the bovine respiratory tract. Vet Med (Praha) 2011;56:28–34.
14. Gourlay RN, Leach RH. A new mycoplasma species isolated from pneumatic lungs of calves (Mycoplasma Dispar Sp. Nov.). J Med Microbiol 1970;3:111–123.
15. Angen O, Thomasen J, Larsen LE, et al. Respiratory disease in calves: Microbiological investigations on trans-tracheally aspirated bronchoalveolar fluid and acute phase protein response. Vet Microbiol 2009;137:165–171.
16. Auto T, Pohjanpaa T, Holopainen R, et al. Etiology of respiratory disease in non-vaccinated, non-mediated calves in rearing herds. Vet Microbiol 2007;119:256–265.
17. Benesi FJ, Bertagnon HG, Wachholz L, et al. Microbiota bacteriana e etiologia da região traqueobronquica de bezerros no período neonatal. Pesq Vet Bras 2013;33:700–704.
18. Gaeta NC, Lima SF, Teixeira AG, et al. Deciphering upper respiratory tract microbiota complexity in healthy calves and calves that develop respiratory disease using shotgun metagenomics. J Dairy Sci 2016;100:1445–1458.
19. Whitford HW, Rosenbusch RF, Lauerman LH. Mycoplasmosis in Animals: Laboratory Diagnosis. Ames: Iowa State University 1994, 150p.
20. Fan HH, Kleven SH, Jackwood MW. Application of polymerase chain reaction with arbitrary primers to strain identification of \textit{Mycoplasma gallisepticum}. Avian Dis 1995;39:729–735.
21. van Kuppevelt FJ, van der Logt JT, Angulo AF, et al. Genus- and species-specific identification of mycoplasmas by 16S rRNA amplification. Appl Environ Microbiol 1992;58:2606–2615.
22. Chávez González YR, Bascuñana CR, Bolsée G, et al. In vitro amplification of the 16S rRNA genes from Mycoplasma bovis and \textit{Mycoplasma agalactiae} by PCR. Vet Microbiol 1995;47:183–190.
23. Marques LM, Buzinhani M, Yamaguti M, et al. Use of a polymerase chain reaction for detection of \textit{Mycoplasma dispar} in the nasal mucus of calves. J Vet Diagn Invest 2007;19:103–106.
24. Dedieu L, Mady V, Lefevre PC. Development of a selective polymerase chain reaction assay for the detection of \textit{Mycoplasma mycoides} subspp. \textit{Mycoplasma} S.C. (Contagious bovine pleuropneumonia agent). Vet Microbiol 1994;42:327–339.
25. Zinka MR. \textit{Mycoplasma} isolated from the respiratory tract of cattle in Bosnia and Herzegovina. An Vet 2012;25:79–83.
26. Thomas A, Ball H, Dizier I, et al. Isolation of mycoplasma species from the lower respiratory tract of healthy cattle and cattle with respiratory disease in Belgium. Vet Rec 2002;151:472–476.
27. Marques LM, Buzinhani M, Yamaguti M, et al. Prevalence of mycoplasmas in the respiratory tracts of calves in Brazil. Vet Rec 2007;161:699–700.
28. Carrington CAP. The Role of Mycoplasma Species in Bovine Respiratory Disease Complex in Feedlot Cattle in South Africa. 284f, Pretoria. Dissertação (Mestrado em Production Animal Studies), University of Pretoria. 2007.

29. Maunsell FP, Donovan GA. Mycoplasma bovis Infections in Young Calves. Vet Clin North Am - Food Animal Pract 2009;25:139–177.

30. Howard CJ, Thomas LH, Parsons KR. Comparative pathogenicity of Mycoplasma bovis and Mycoplasma dispar for the respiratory tract of calves. Israel J Med Sci 1987;23:621–624.

31. Oliveira BAFD, Gueta NC, Ribeiro BLM, et al. Determination of bacterial aetiologic factor on tracheobronchial lavage in relation to clinical signs of bovine respiratory disease. J Med Microbiol 2016;65:1137–1142.

32. Gevaert D. The importance of Mycoplasma bovis in bovine respiratory disease. Tijdschr Diergeneesk. 2006;131:124–126.

33. Akan M, Babacan O, Torun E, et al. Diagnosis of Mycoplasma bovis infection in cattle by ELISA and PCR. KAFKAS Univ Vet Fak Derg 2014;20:249–252.

34. Riberio OC. Experimental Infection of Calves with Mycoplasma Dispar. 162f. Ames, IA, USA. Tese (Doutorado em Patologia Veterinária), Iowa State University; 1979.