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MICROBIOLOGY

Epidemiology of *Mycoplasma agalactiae* and *Mycoplasma mycoides* cluster in flocks of northeastern Brazil

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ABSTRACT: *The present study aimed to investigate contagious agalactia (CA) in flocks from Pernambuco State. The study involved 225 goats and 63 ewes; 288 milk samples and 100 vaginal swabs were collected in total. The PCR assays were carried out using specific primers to Mycoplasma agalactiae and the Mycoplasma mycoides cluster. Among the goat's milk samples,12.0% (27/225) were positive for Mycoplasma agalactiae DNA, while 5.3% (12/225) contained the Mycoplasma mycoides cluster. Of the vaginal swabs taken from goats, 15.4% (12/78) were positive for Mycoplasma agalactiae DNA and 3.8% (3/78) contained the Mycoplasma mycoides cluster. In the case of ewes, 4.3% (1/23) of the milk samples contained Mycoplasma agalactiae DNA, and 7.5% (3/40) were positive for the Mycoplasma mycoides cluster. Vaginal swabs taken from sheep´s were negative. Analysis of risk factors for mycoplasmosis, showed that goats and sheep flocks on the extensive breeding system are more likely to have mycoplasmosis than those on the intensive breeding system (odds ratio (OR) 6.2; p=0.004); meat goat and sheep flocks are more likely to have infection compared to dairy flocks (OR 4.8; p=0.011); unclean animal housing increases the chances of infection (OR 5.0; p=0.031) and not performing quarantine increases the chances of mycoplasmosis (OR 4.6; p=0.042). Based on these findings we conclude that CA syndrome in the semiarid region of Pernambuco state can be associated with Mycoplasma agalactiae and Mycoplasma mycoides cluster.*
Key words: *contagious agalactia, flocks, diagnostic, epidemiology, risk factor.*

Epidemiologia de *Mycoplasma agalactiae* e *Mycoplasma mycoides* cluster em rebanhos do nordeste do Brasil

RESUMO: *O objetivo deste estudo foi investigar a Agalaxia contagiosa em rebanhos do estado de Pernambuco. Foram examinadas 225 cabras e 63 ovelhas, das quais foram colhidas 288 amostras de leite e 100 suabes vaginais. Foram realizadas reações da PCR com iniciadores específicos para Mycoplasma agalactiae e Mycoplasma mycoides cluster. A frequência total de Mycoplasma agalactiae em amostras de leite caprino foi de 12,0% (27/225) e de 5,3% (12/225) para Mycoplasma mycoides cluster. Dos suabes vaginais de cabras as frequências detectadas na PCR foram de 15,4% (12/78) para Mycoplasma agalactiaee 3,8% (3/78) para Mycoplasma mycoides cluster. Em leite de ovelhas a frequência de Ma foi de 4.3% (1/23) e de 7,5% (3/40) para Mycoplasma mycoides cluster. Na análise dos fatores de risco para micoplasmoses verificou-se que rebanhos de caprinos e ovinos mantidos no sistema extensivo são mais prováveis de adquirir micoplasmose quando comparados com o sistema intensivo (odds ratio (OR) 6,2; p=0,004); rebanhos de caprinos e ovinos de corte são mais prováveis de adquirir micoplamsose do que rebanhos de leite (OR 4,8; p=0,011); não realizar limpeza das instalações aumenta as chances de infecção (OR 5,0; p=0,031); não realizar quarentena aumenta as chances das micoplasmoses estudadas (OR 4,6; p=0,042). Conclui-se que M. agalactiae e Mycoplasma mycoides cluster estão envolvidos na síndrome de CA em rebanhos de caprinos e ovinos do semiárido pernambucano.*
Palavra-chave: *agalaxia contagiosa, rebanhos, diagnóstico, epidemiologa, fatores de risco.*

INTRODUCTION

Contagious agalactia (CA) is an infectious disease that affects goats and sheep. It is characterized by mastitis-agalactia, arthritis, and keratoconjunctivitis. Outbreaks of CA have been reported in several wild ruminants (CHAZEL et al., 2010; OSTROWSKI et al., 2011). The disease is caused by *Mycoplasma agalactiae (Ma)*, as well as by several species in the *Mycoplasma mycoides* (*M. mycoides*) cluster; namely, *Mycoplasma capricolum* subsp. *capricolum* (*Mcc*), *Mycoplasma*

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mycoides subsp. *capri* (*Mmc*), and *Mycoplasma putrefaciens* (*Mp*). All of these pathogens cause indistinguishable clinical symptoms (OIE, 2013). Of these, *Mp* was included in the phylogenetic *M. mycoides* cluster by MANSO-SILVÁN et al. (2007). The *M. mycoides* cluster has complex taxonomy and includes the causative agents of contagious caprine pleuropneumonia and contagious bovine pleuropneumonia. In addition, it encompasses the bovine pathogen *M. leachii* and the small ruminant pathogens *Mmc* and *Mcc* (FISCHER et al., 2012).

The *Ma* primarily affects the mammary glands, eyes, joints, and less frequently the respiratory tract. In contrast, the other species mentioned are primarily related to respiratory diseases (NICHOLAS, 2002). Congenital infections have been reported in newborn goatling in Brazil, confirming that *Ma* is transmitted across the placenta (SILVA et al., 2014). Furthermore, several authors have suggested that *Ma* in goats is transmitted venereally (GIL et al., 2003; AMORES et al., 2011; GÓMEZ MARTÍN et al., 2012). In sheep, *Ma* is the most common causative agent of CA; although, *Mmc* also occurs sporadically. Conversely, goat CA is more complex, four species can cause the disease (*Ma, Mcc, Mmc* and *Mp*), and mixed infections have been reported in the Mediterranean region (GÓMEZ MARTÍN et al., 2012; GÓMEZ MARTÍN et al., 2013). More specifically, the *Mmc* and *Mcc* species cause 'MAKePS' syndrome, (mastitis, arthritis, keratitis, pneumonia, and septicemia), while *Mp* mainly causes mastitis and arthritis (PEYRAUD et al., 2003).

The epidemiology and geographical distribution of CA remain unclear in South America. In Brazil specifically, only a few authors have reported CA outbreaks in goats and sheep (NASCIMENTO et al., 1986; AZEVEDO et al., 2006). Nonetheless, as control measures have failed, the disease has spread to many regions of the country. The present study constitutes an epidemiological and molecular investigation of CA causing agents in semiarid mesoregions in the state of Pernambuco, northeastern Brazil.

MATERIALS AND METHODS

This investigation was conducted across eleven properties in semiarid regions with a high concentration of goat and sheep stocks. The target districts were as follows: Serra Talhada (two goat farms, A and B, and one sheep farm, C), Sertânia (five farms of Saanen and Toggenburg dairy goats maintained in intensive production management, D, E, F, G, and H), Custódia (one goat farm, I), and Floresta (one sheep farm, J and one goat farm, K).

After visual inspection of the animals, 225 goats' and 63 ewes' milk samples and 100 vaginal swabs were collected, regardless of the animals' clinical symptoms. To collect the milk samples, the ostium ceiling was flushed with water and soap, dried with wipes, and sterilized with 70% alcohol. Next, numbered, sterile vials were used to collect 5mL of milk from each mammary gland. In addition, 100 vaginal swabs in total were collected from 78 goats and 22 ewes using sterile swabs rubbed on the lateral and internal walls of the vagina. These were then stored in tubes containing 2mL of sterile phosphate-buffered saline (pH 7.2).

The milk and vaginal swabs were then refrigerated at 4ºC and transported to the Laboratory of Infectious Diseases (LDIC/DMV/UFRPE), where they were submitted to DNA extraction using commercially available kits (Wizard SV Genomic DNA purification System®; Promega Corporation, Madison, WI, USA; Ref. A2361 for the milk samples and Ref. A1125 for the vaginal swabs). These kits were used according to the manufacturer's instructions; although, some adjustments were made. Quantity and quality of the extracted DNA were evaluated using an automatic quantifier (Multiscan Go®; ThermoScientific). Next, PCR assays were carried outusing specific primers for *Ma* (FS1:5'-AAAGGTGCTTGAGAAATGCC-3'and FS2: 5'-GTTGCAGAAGAAAGTCCAATCA-3', which amplify a 375-bp fragment) and for the *Mycoplasma mycoides* cluster (F-REAP:5'-GAAACGAAAGATAATACCGCATGTAG-3'and R-REAP:5'-CCACTTGTGCGGGTCCCCGTC-3', which amplify a 785-bp fragment).

The PCR reaction used a thermal profile that has been previously described, with some adjustments (TOLA et al., 1997; PERSSON et al., 1999). A reaction mix was prepared containing 5μL of DNA template, 30pmol of each primer, 6.25μL of GoTaq®Green Master Mix (Promega® Corporation, Madison, WI, USA; Ref. M7122) and Milli-Q ultrapure water up to 25μL, in accordance with the manufacturer's instructions. *Mycoplasma mycoides* subsp.*capri* (GM12) DNA was used as a positive control in the *M. mycoides* cluster reactions, as well as in the reactions for a *Mycoplasma agalactiae* strain isolated in Paraiba State (BrPB01; Gen Bank No.JQ612164;0.8ng/μL). Reactions were carried out in a thermocycler (Bioer XP Thermal Cycler®;

Bioer Technology Corporation Ltda., Hangzhou, China). Products were then analyzed in 1.5% electrophoresis gel, and the amplicons visualized in a transilluminator (L-*Pix*; LocusBiotechnology®) and photographed.

The genomic DNA samples obtained in the PCR were purified using a commercial kit, MegaQuick-spin™ Total Fragment DNA Purification Kit (Intron Biotechnology®, Korea). Subsequently the DNA was quantified using an automatic quantifier for quality and quantity measurement. Purified products after amplification were bidirectionally sequenced using the BigDyeTerminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA), according to the manufacturer's instructions. The primers used for the sequencing were the same as used in the amplification step.

Milk and vaginal swab samples that were positive for mycoplasma DNA in the PCR were processed for mycoplasma isolation. In the case of milk samples, 2mL was sterilized using a syringe coupled with a membrane filter (0.45µM), and 100µL of the filtrate was diluted to concentrations of 10^{-1} to 10^{-5} and inoculated onto liquid and solid modified Hayflick's medium. These samples were incubated at 37°C for 21 days, and the agar plates were then placed in a microaerophilic jar. Growth in the plates was verified using a stereomicroscope (80× magnification). Mollicute isolates were confirmed using a Dienes probe, and *Mycoplasma* genera were identified with a digitonin sensitivity test (WHITFORD et al., 1994; RAZIN & TULLY, 1996).

Epidemiological questionnaires were used to obtain information about the type of farming and management practices and thus identify possible risk factors in each herd. The questionnaires were delivered by a single trained investigator and answered by personnel who could provide information about the animals'nutrition, reproduction, and milking system. Variables investigated and their respective answer categories were as follows: consortium creation (yes/no); herd type (dairy/dairy and meat); wetlands on the property (yes/no); flooded areas on surrounding properties (yes/no); presence of hematophagous insects (yes/no); insect control (yes/no); presence of quarantine (yes/no); reproductive management (natural breeding/artificial insemination/both). Univariate analysis of infection-associated risk factors was performed using the Pearson chi-square test (χ^2) or Fisher's exact test. A logistic regression analysis was then performed, which considered the "Gold standard" PCR result for mycoplasmas (*Ma* or *M. mycoides* cluster) as the dependent variable. Independent or explanatory variables with a p-value <0.20 were considered in the model, so that no possible event risk factors were excluded from the analysis (HOSMER & LEMESHOW, 1989). EpiInfo7™ software was used to perform statistical calculations; the significance level was set at 5.0%.

RESULTS

In this study, *Mycoplasmataceae* were detected using PCR in farms from semiarid mesoregions. Figure 1 shows gel electrophoresis of PCR results for *Ma* and *M. mycoides* cluster. We found that 18.2% (41/225) of goats and 6.3% (4/63) of sheep were positive for *Mycoplasmataceae* DNA. On each farm, the *Ma* results were as follows: A—63.6%, B—17.6%, C—4.0%, and K—8.3%. The *M. mycoides* cluster results on the individual farms were: E—25.0, H—7.7, I—11.7, J—7.5, and K—10.0. Figure 2 shows the frequency of *Ma* and *M. mycoides* cluster on each farm, as detected by PCR analysis.

Throughout all districts, the frequencies of *Ma* and *M. mycoides* cluster in goats' milk samples were 12.0% and 5.3%, respectively; table 1 shows the results in the individual districts. In the goat vaginal swabs, the frequency of *Ma* was 15.4% (12/78), while that of the *M. mycoides* cluster was 3.8% (3/78). The highest *Ma* frequency in goat vaginal swabs (30.0%) occurred in the Serra Talhada district. In ewes' milk, *Ma* was detected in 4.3% (1/23) of samples, while the *M. mycoides* cluster was found in 7.5% (3/40). Ewes' vaginal swabs were all negative for *Mycoplasmataceae* in the PCR analysis. Among farms that shared an owner, some signals of CA were reported, such as polyarthritis (19.2%), sudden drop in milk production (11.8%), and reproductive disorders (17.9%). It was possible to confirm the viability of the bacteria, by isolation in milk, where by mollicutes grew in 26.7% of the goats' milk samples.

In the subsequent analysis, the main risk factor for CA was the extensive system of goat breeding, which carried a higher risk of mycoplasmosis than the intensive system (OR = 6.2; p=0.004). Furthermore, meat flocks were more likely to carry infection than dairy flocks (OR = 4.8; p=0.011). Failure to clean the animals' housing increases the risk of infection (OR = 5.0; p=0.031), as does failure to perform quarantine (OR = 4.6; p=0.042; Table 2).

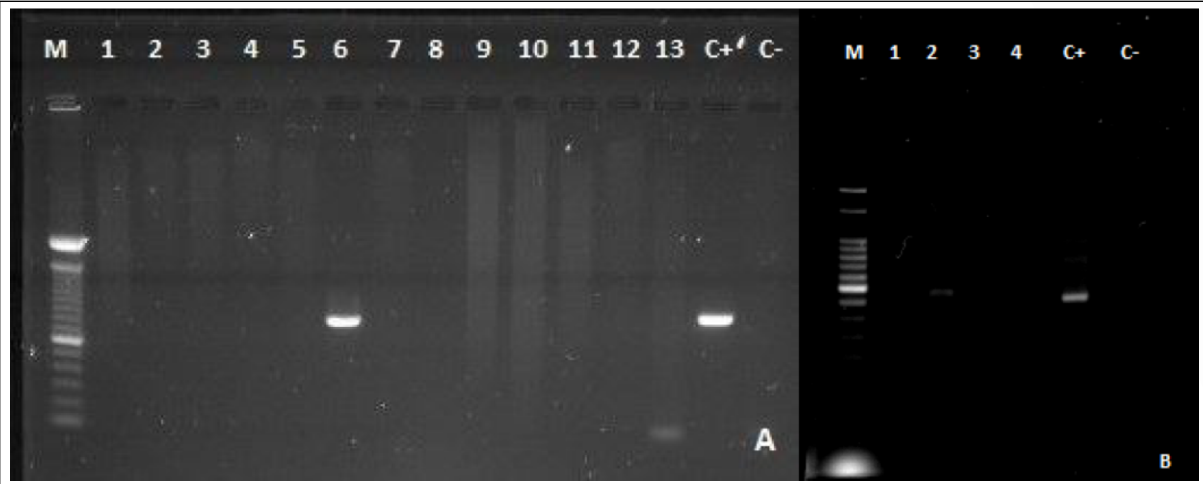

Figure 1 - Results of PCR for the *Mycoplasma mycoides* cluster and *Mycoplasma agalactiae* in milk samples from goats. A) Lane 6: Positive samples for the *Mycoplasma mycoides* cluster with an amplicon 785bp. B) Lane 2: Positive samples *Mycoplasma agalactiae* with an amplicon 375bp. Lane (C-) negative control and Lane (C+) Positive control; Lane M, molecular size marker (100bp DNA *Ladder*, amplicon size 100-1kb).

DISCUSSION

CA is a significant infectious disease among goat and sheep flocks, which limits production on both dairy and meat farms. In the present study, the prevalence of CA in goats, as expected, corroborated previously published data from endemic areas of northeastern of Brazil, which range from 20% to 56.43% of infection (BANDEIRA et al., 2008). In contrast, using serological analysis during CA outbreaks among goats in the Paraiba State, CAMPOS et al. (2009) reported a higher prevalence of 83.2%. In ewes and lambs; however, the prevalence of infection varies. For instance, in a CA outbreak in Paraiba State, the morbidity rate was of 26% in goats and 49% in lambs, with a total prevalence of 100% (AZEVEDO et al., 2006). In France, CHAZEL et al., (2010) reported agalactia is most often caused by *Mmc, Mcc*, and *Mp* in goats, whilst in sheep, *Ma* is the most common causative species. In Spain, ARIZA-MIGUEL et al. (2012) reported that the prevalence of CA in sheep varied from 50% to 100% on different farms; in Jordan, AL-MOMANI et al. (2008) reported a prevalence of 39% in sheep and 36% in goats.

Distribution of *Ma* among dairy sheep and goats varies in different locations. In Spanish dairy sheep farms, *Ma* is the most common causative factor, while the *M. mycoides* cluster is reported most often in goat flocks (ARIZA-MIGUEL et al., 2012). In the present study, we reported that both *Ma* and the *M. mycoides* cluster circulate freely in goat and sheep flocks. More specifically, conditions that favor spread include keeping animals in mixed flocks, poor sanitary conditions, and inadequate facilities. For instance, the high prevalence of CA in farm A was likely due to poor sanitary management, which contributes to the spread of *Ma* and *M. mycoides* cluster. On the same farm, the farmer had a history of managing flocks with CA. Among all the farms investigated, the most common causes of CA among meat goats and sheep were poor sanitary conditions and inadequate facilities. In farms that used an intensive breeding system, the probable prevalence of the *M. mycoides* cluster was 25%, and it was common to exhibit and trade animals without disease control.

AL-MOMANI et al. (2008; 2011) reported that the seroprevalence values of *Ma* among sheep, goats, and mixed flocks are 25%, 21%, and 30%; those of the *Mmc* were 32%, 38%, and 34% in the same study, suggesting that these mycoplasmas circulate widely among flocks of sheep and goats that are kept together in mixed farms. Similar circumstances occur in the semiarid regions of northeastern Brazil. Using PCR, ALVES et al. (2013) reported *Ma* frequencies of 17.9 in goat semen and 3.7% in goats' milk. In the present study we identified a higher frequency of 30% among milk samples of goats.

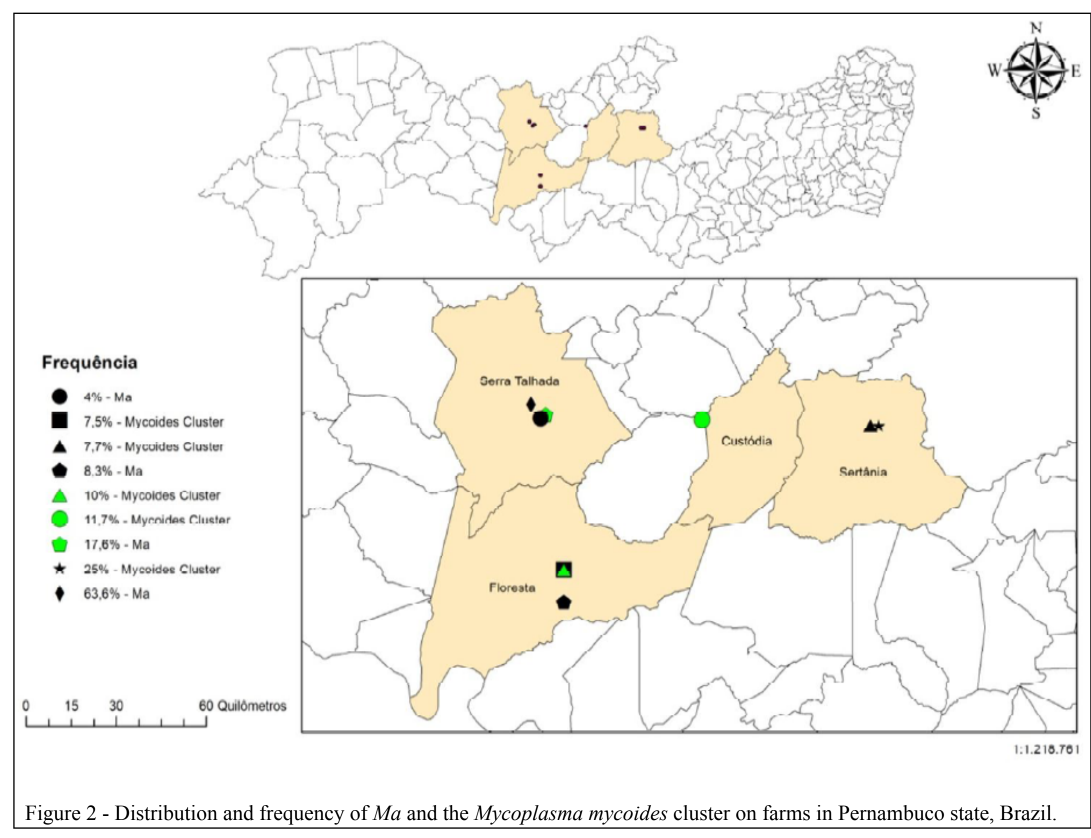

Figure 2 - Distribution and frequency of *Ma* and the *Mycoplasma mycoides* cluster on farms in Pernambuco state, Brazil.

On a separate note, outbreaks of respiratory syndrome do occur among goats in Brazil. These outbreaks are associated with mastitis, arthritis, and keratoconjunctivitis caused by *Mmc* (NASCIMENTO et al., 1986). The present study corroborated these results by identifying *Mycoplasma mycoides* cluster in areas endemic for CA. Furthermore, the risk factors for *Ma* and the *M.mycoides* cluster were as follows: extensive breeding system, type of flock (meat), and failure to clean animal housing or perform quarantine. In their study of risk factors for *Ma* seroprevalence among sheep and goats, AL-MOMANI et al. (2008) demonstrated that only three variables increased *Ma* risk: use of outside rams, improper cleaning of the milking utensils, and separation of young from their mother. Failure to perform quarantine increased the risk of mycoplasmosis. NICHOLAS (1999) reported a similar correlation in the use of rams from other flocks for breeding which was accompanied by increasing seroprevalence of *Ma.*

Similarly, cleaning of milking utensils should decrease infection by *Ma*; accordingly, the

Table 1 - Results of PCR for *Ma* and the *Mycoplasma mycoides* cluster in goats from northeastern Brazil.

Districts	*Ma*	Milk	(Goat)	Vaginal swab
		Myc. mycoides Cluster	*Ma*	*Myc. mycoides Cluster*
Custódia (n=34)	0	3/34 (8.8)	0	0
Floresta (n=60)	5/60 (8.3)	6/60 (10.0)	0	0
Serra Talhada (n=73)	22/73 (30.0)	0	12/73 (16.4)	3/73 (4.1)
Sertânia (n=58)	0	3/58 (5.2)	0	0
Total	27/225 (12.0)	12/225 (5.3)	12/78 (15.4)	3/78 (3.8)

Table 2 - Risk factors for mycoplasmosis in the goat and sheep flocks investigated.

Variables	N	Mycoplasmosis	P value logistic regression	OR (I.C. 95%)	P value
		PCR			
---Method of rearing---					
Intensive	58	3 (5.2%)		-	
Semi-intensive	60	8 (13.3%)	0.003[(A)*]	2.8 (0.7-11.2)	0.140
Extensive	107	27 (25.2%)		6.2 (1.8-21.4)	0.004*
---Type of farm---					
Meat	167	35 (20.9%)	0.004[(B)*]	4.8 (1.4-14.4)	0.011*
Milk	58	3 (5.2%)			
---Flock size---					
< 50 animals	54	15 (27.8%)			
Between 51 - 100 animals	26	2 (7.7%)	0.034[(B)*]		
Above >101 animals	145	21 (14.5%)			
---Waterers and feeders common---					
Yes	199	36 (18.1%)	0.266[(B)]		
No	26	2 (7.7%)			
---Insects control---					
Yes	26	2 (7.7%)	0.266[(B)]		
No	199	36 (18.1%)			
---Cleaning of premises---					
Weekly	40	2 (5.0%)		-	
Monthly	18	1 (5.5%)	0.022[(A)*]	1.1 (0.1-13.2)	0.929
No	167	35 (20.9%)		5.0 (1.1-21.9)	0.031*
---Quarantine---					
Yes	40	2 (5.0%)	0.033[(B)*]		
No	185	36 (19.4%)		4.6 (1.0-19.9)	0.042*
---Animals with mastitis remain with others animals---					
Yes	199	36 (18.1%)	0.266[(B)]		
No	26	2 (7.7%)			
---Fate of sick animals---					
Slaughter	134	27 (20.1%)			
Commerce	14	0 (0.0%)	0.120[(A)]		
Treatment	77	11 (14.3%)			

[(A)]Chi-square test;[(B)] Fisher´s exact test; N – Total samples; OR – Odds Ratio; I.C. – Confidence Interval;[1] Database used: (n=80); *Significant association to the level of 5.0%.

present study demonstrated that failure to clean the animal housing was a risk factor for CA. AL-MOMANI et al. (2008) reported that such improper practices may disseminate *Ma* and increased the number of infected animals in the flock. According to AL-MOMANI et al. (2010), the risk factors for *Mmc* were exacerbated by concentrated grazing supplements, *Mmc* seropositivity, and stress caused by changes in husbandry, nutrition, and climate, which is seen as a major factor among outbreaks in adults.

In the dry season, poor sanitary management and nutrition may promote the spread of *Ma* and the *M. mycoides* cluster by inhaled aerosol among sheep and goats, particularly if the animals are kept together (MADANAT et al., 2001;AL-MOMANI et al., 2008). A similar situation may occur in semiarid regions of northeastern Brazil. Thus, *Ma* and the *M. mycoides* cluster should be monitored in this group in particular, as these infections are important for animal health and publications. Moreover, *Ma* and *M. mycoides* cluster species cause serious, unexpected economic losses to northeastern Brazil.

CONCLUSION

In this context, it was possible to conclude that *Ma* and *M. mycoides* cluster species occur in high rates in semiarid areas of Pernambuco State.

Inadequate facilities and extensive breeding enhance the risk of CA and other mycoplasmosis.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This research was submitted to the ethics committee for animal research (CEUA-UFRPE; No.23082.007834/2015-73).

DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflicts of interest.

REFERENCES

AL-MOMANI, W. et al. Risk factors associated with *Mycoplasma agalactiae* infection of small ruminants in northern Jordan. **Preventive Veterinary Medicine**, v.83, p.1-10, 2008. Available from: <https://www.sciencedirect.com/science/article/pii/S0167587707001833?via%3Dihub>. Accessed: Oct. 5, 2016. doi: 10.1016/j.prevetmed.2007.08.003.

AL-MOMANI, W. et al. Seroprevalence of and risk factors for *Mycoplasma mycoides* subspecies *capri* infection in small ruminants in Northern Jordan. **Tropical Animal Health and Production**, 2010. Available from: <https://www.researchgate.net/publication/47500597>. Accessed: Oct. 6, 2016. doi: 10.1007/s11250-010-9717-9.

ALVES, B.H.L.S. et al. *Mycoplasma agalactiae* in semen and milk of goat from Pernambuco State, Brazil. **Pesquisa Veterinária Brasileira**, v.33, p.1309-1312, 2013. Available from: <http://www.scielo.br/scielo.php?pid=S0100-736X2013001100004&script=sci_arttext&tlng=es>. Accessed: Oct. 10, 2016: doi: 10.1590/S0100-736X2013001100004.

AMORES, A.J. et al. Presence of contagious agalactia causing mycoplasmas in Spanish goat artificial insemination centres. **Theriogenology**, v.75, p.1265-1270, 2011. Available from: <http://doi:10.1016/j.theriogenology.2010.11.040>. Accessed: Aug. 5, 2013. doi: 10.1016/j.theriogenology.2010.11.040.

ARIZA-MIGUEL, J. et al. A survey of *Mycoplasma agalactiae* in dairy sheep farms in Spain. **BMC Veterinary Research**, v.8, p.171, 2012. Available from: <https://bmcvetres.biomedcentral.com/articles/10.1186/1746-6148-8-171>. Accessed: Oct. 05, 2016. doi: 10.1186/1746-6148-8-171.

AZEVEDO, A.E.O. et al. Contagious agalactia by *Mycoplasma agalactiae* in small ruminants in Brazil: first report. **Brazilian Journal Microbiology**, v.37, p.576-581, 2006. Available from: <http://www.scielo.br/scielo.php?pid=S1517-83822006000400033&script=sci_arttext&tlng=pt>. Accessed: Feb. 06, 2017. doi: 10.1590/S1517-83822006000400033.

BANDEIRA, D.A. et al. Infection by *Mycoplasma agalactiae* in dairy goat herds in the microregionsof Cariri in ParaíbaState. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.60, p.1255-1258, 2008. Available from: <http://www.scielo.br/scielo.php?pid=S0102-09352008000500031&script=sci_arttext&tlng=pt>. Accessed: Oct. 05, 2016. doi: 10.1590/S0102-09352008000500031.

CAMPOS, A.C. et al. ELISA protein G for the diagnosticof contagious agalactia in small ruminants. **Small Ruminant Research**, v.84, p.70-75, 2009. Available from: <http://www.elsevier.com/locate/smallrumres>. Accessed: Jun. 26, 2017.doi: 10.1016/j.smallrumres.2009.06.006.

CHAZEL, A.M. et al. Mycoplasmoses of ruminants in France: recent data from the national surveillance network. **BMC Veterinary Research**, v.6, p.32, 2010. Available from: <https://bmcvetres.biomedcentral.com/articles/10.1186/1746-6148-6-32>. Accessed: Jun. 24, 2017. doi: 10.1186/1746-6148-6-32.

FISCHER, A. et al. The origin of the '*Mycoplasma mycoides Cluster*' coincides with domestication of ruminants. **PloS ONE**, v.7, p.e36150, 2012. Available from: <http://www. doi:10.1371/journal.pone.0036150>. Accessed: Oct. 06, 2017. doi: 10.1371/journal.pone.0036150.

GIL, A.M.C. et al. Genital lesions in an outbreak of caprine contagious agalactia caused by *Mycoplasma agalactiae* and *Mycoplasma putrefaciens*. **Journal Veterinary Medicine B**,v.50, p.484-487, 2003. Available from: <http://onlinelibrary.wiley.com/doi/10.1046/j.0931-1793.2003.00709.x/full>. Accessed:Jun. 24, 2017. doi 10.1046/j.0931-1793.2003.00709.x.

GÓMEZ MARTÍN, A.A. et al. Controlling contagious agalactia in artificial insemination centers for goats and detection of *Mycoplasma mycoides* subspecies *capri* in semen. **Theriogenology**, v.77, p.1252-1256, 2012. Available from: <http://doi:10.1016/j.theriogenology.2011.10.006>. Accessed: Sept. 27, 2017. doi: 10.1016/j.theriogenology.2011.10.006.

GÓMEZ-MARTÍN, A.A. et al. Contagious agalactia due to *Mycoplasma* spp. in small dairy ruminants: Epidemiology and prospects for diagnosis and control. **Veterinary Journal**, v.198, p.48-56, 2013. Available from: <http://dx.doi.org/10.1016/j.tvjl.2013.04.015>. Accessed: Sept. 27, 2017. doi: 10.1016/j.tvjl.2013.04.015.

HOSMER, A.D.W., LEMESHOW, S. **Applied Logistic Regression**. NewYork: Sons John Wiley, 1989.

MADANAT, A.A. et al. Contagious agalactia of sheep and goats: A review. **Acta Veterinary BRNO**, v.70, p.403-412, 2001. Available from: <http://www.vfu.cz/acta-vet/actavet.htm>. Accessed: Oct. 18, 2016. doi: 10.2754/avb200170040403.

MANSO-SILVÁN, L. et al. Phylogeny of the *Mycoplasma mycoides* cluster based on analysis of five conserved protein-coding sequences and possible implications for the taxonomy of the group. **International Journal of Systematic and Evolutionary Microbiology**, v.57, p.2247-2258, 2007. Available from: <http://www.microbiologyresearch.org/docserver/fulltext/ijsem/57/10/2247.pdf?expires=1520354310&id=id&accname=guest&checksum=BA7175DD55786803D444CAC48863D39E>. Accessed: Jan. 01, 2006. doi: 10.1099/ijs.0.64918-0.

NASCIMENTO, E.R. et al. Isolation of *Mycoplasma mycoides* from outbreaks of caprine mycoplasmosis in Brazil. **British Veterinary Journal**, v.142, p.246-257, 1986. Available from: <https://www.

sciencedirect.com/science/article/pii/0007193586900680>. Accessed: Oct. 05, 2016. doi: 10.1016/0007-1935(86)90068-0.

NICHOLAS, R.A. Improvements in the diagnosis and control of diseases of small ruminants caused by mycoplasmas. **Small Ruminant Research**, v.45, p.145-149, 2002. Available from: <http://www.smallruminantresearch.com/article/S0921-4488(02)00095-0/pdf>. Accessed: Jun. 24, 2017. doi: 10.1016/S0921-4488(02)00095-0.

NICHOLAS,R.A.J. Isolation of mycoplasma ovine/caprine serogroup 11 from infertile sheep in Britain. **Veterinary Record**, v.145(15), p.434-435, 1999. Available from: <https://www.cabdirect.org/cabdirect/abstract/19992216189>. Accessed: Jun. 24, 2017. doi: 10.1136/vr.145.15.434.

OIE (ORGANIZATION INTERNATIONAL OF EPIZOOTIES). **Terrestrial Manual: ContagiousAgalactia**. 2013. Available from: <http://www.oie.int/en/international-standard-setting/terrestrial-manual/access-online>. Accessed: Jul. 21, 2016.

OSTROWSKI, A.S. et al. Fatal outbreak of *Mycoplasma capricolum* pneumonia in endangered markhors. **Emerging Infectious Diseases**, v.17, p.2338-2341, 2011. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3311196/pdf/11-0187_finalD.pdf>. Accessed: Jul. 21, 2016. doi: 10.3201/eid1712.110187.

PERSSON, A. et al. Diagnosis of contagious bovine pleuropneumonia by PCR laser-induced fluorescence and PCR restriction endonuclease analysis based on the 16SrRNA genes of *Mycoplasma mycoides* subsp. *mycoides* SC. **Journal Clinical Microbiology**, v.37, p.3815-3821, 1999. Available from: <http://jcm.asm.org/content/37/12/3815.full.pdf+html>. Accessed: Oct. 5, 2016. doi: jcm.asm.org/content/37/12/3815.

PEYRAUD, A. et al. A specific PCR for the detection of *Mycoplasma putrefaciens*, one of the agents of the contagious agalactia syndrome of goats. **Molecular and Cellular Probes**, v.17, p.289-294, 2003. Available from: <https://www.sciencedirect.com/science/article/pii/S0890850803000732>. Accessed: Jan. 01, 2006. doi: 10.1016/j.mcp.2003.07.006.

RAZIN, S.;TULLY, J.G. **Molecular and diagnostic Procedures in Mycoplasmology**. California:Academic Press, 1996.

SILVA, A.N.S. et al. Congenital infection by *Mycoplasma agalactiae* in goat kids. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.66, p.631-634, 2014. Available from: <http://www.scielo.br/scielo.php?pid=S0102-09352014000200044&script=sci_arttext&tlng=pt>. Accessed: Feb. 27, 2015. doi: 10.1590/1678-41626625

TOLA, A. et al. Detection of *Mycoplasma agalactiae* in sheep milk samples by polymerase chain reaction. **Veterinary Microbiology**, v.54, p.17–22, 1997. Available from: <https://www.sciencedirect.com/science/article/pii/S0378113596012692>. Accessed: Jan. 01, 2006. doi: 10.1016/S0378-1135(96)01269-2.

WHITFORD, H.W. et al. **Mycoplasmosis in Animals: Laboratory Diagnosis**. Iowa S.:AmesUniversityPress, 1994.