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Seroprevalence and risk factors associated with *Chlamydophila abortus* infection in dairy goats in the Northeast of Brazil[1]

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Few data are available on the prevalence and risk factors of *Chlamydophila abortus* infection in goats in Brazil. A cross-sectional study was carried out to determine the flock-level prevalence of *C. abortus* infection in goats from the semiarid region of the Paraíba State, Northeast region of Brazil, as well as to identify risk factors associated with the infection. Flocks were randomly selected and a pre-established number of female goats ≥ 12 mo old were sampled in each of these flocks. A total of 975 serum samples from 110 flocks were collected, and structured questionnaire focusing on risk factors for *C. abortus* infection was given to each farmer at the time of blood collection. For the serological diagnosis the complement fixation test (CFT) using *C. abortus* S26/3 strain as antigen was performed. The flock-level factors for *C. abortus* prevalence were tested using multivariate logistic regression model. Fifty-five flocks out of 110 presented at least one seropositive animal with an overall prevalence of 50.0% (95%; CI: 40.3%, 59.7%). Ninety-one out of 975 dairy goats examined were seropositive with titers ≥32, resulting in a frequency of 9.3%. Lend buck for breeding (odds ratio = 2.35; 95% CI: 1.04-5.33) and history of abortions (odds ratio = 3.06; 95% CI: 1.37-6.80) were associated with increased flock prevalence.

INDEX TERMS: *Chlamydophila abortus*, prevalence, flock-level risk factors, small ruminants, Brazil.

RESUMO.- [**Soroprevalência e fatores de risco associados com a infecção por *Chlamydophila abortus* em caprinos leiteiros do Nordeste brasileiro.**] São escassos os trabalhos publicados sobre a prevalência e fatores de risco associados à infecção por *Chlamydophila abortus* em caprinos no Brasil. Foi conduzido um estudo transversal para determinar a prevalência de rebanhos positivos para a infecção por *C. abortus* em caprinos do semiárido do Estado da Paraíba, Nordeste do Brasil, bem como identificar os fatores de risco associados com a infecção. Os rebanhos foram selecionados aleatoriamente e um número pré-estabelecido de cabras com idade ≥12 meses foi amostrado por rebanho. No total, foi colhido sangue de 975 animais procedentes de 110 rebanhos, e no momento da colheita foi aplicado um questionário epidemiológico a cada proprietário. Para o diagnóstico sorológico foi utilizado o teste de fixação de complemento (FC) usando a estirpe de *C. abortus* S26/3 como antígeno. Os fatores de risco para a prevalência de *C. abortus* em nível de rebanho foram testados com o uso de modelo de regressão logística multivariada. Cinquenta e cinco rebanhos dos 110 analisados apresentaram pelo menos um animal soropositivo, com uma prevalência de 50,0% (IC 95%: 40,3-59,7%). Noventa e um animais entre os 975 examinados foram soropositivos com título ≥32, re-

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sultando em uma frequência de 9,3%. Compartilhar reprodutores (*odds ratio* = 2,35; IC 95%: 1,04-5,33) e histórico de abortamentos (*odds ratio* = 3,06; IC 95%: 1,37-6,80) foram associados com o aumento da prevalência de rebanhos.

TERMOS DE INDEXAÇÃO: *Chlamydophila abortus*, prevalência, fatores de risco em nível de rebanho, pequenos ruminantes, Brasil.

INTRODUCTION

Chlamydophilosis is a bacterial infection caused by *Chlamydophila abortus*, a recognized zoonotic pathogen that infects farm animals and has been implicated as a major cause of abortions in goats and sheep (Sharma et al. 2003, Masala et al. 2007). In sheep, the agent causes the ovine enzootic abortion (OEA), which is characterized by abortion during late pregnancy and premature birth of weak lambs (Marsilio et al. 2005). In Hungary and USA, *C. abortus* has been incriminated as the main cause of abortion in goats (Moeller 2001, Szeredi & Bacsadi 2002). In addition to the economic importance in the sheep and goat industry, *C. abortus* induces abortions in humans as a result of contact with aborting sheep or goats (Pospischil et al. 2002, Aitken & Longbottom 2007).

The development of clinical signals of chlamydophilosis depends on the time period of infection. Sheep and goats infected 5-6 weeks before giving birth can develop the clinical disease during their current gestation (Morgan et al. 1988). Animals infected during the last four weeks of gestation can develop a latent infection; clinical signs then appear during the next gestation. It has been shown that the reproductive organs of ewes and goats suffering from a latent infection can contain *C. abortus* even after more than 3 years after the infection (Morgan et al. 1988). Lambs and kids delivered by infected animals are generally weak and die a few days after birth (Al-Qudah et al. 2004).

In Brazil, serological assessments of the *C. abortus* in goats are rare. Piatti et al. (2006) conducted a serological work in goats and sheep from the states of São Paulo, Mato Grosso, Minas Gerais and Bahia, and found a prevalence of 12.0% for goats, and all sheep were negative. Pereira et al. (2009) conducted a seroepidemiological study aiming to determine the occurrence of infection and identify risk factors in sheep and goats in Pernambuco State, and reported frequencies of seropositivity of 8.1% in goats and 12.0% in sheep, and predominance of pure breeds (odds ratio = 6.65) and intensive management (odds ratio = 4.18) were identified as risk factors for infection in goats.

Goats are economically important in many countries, including Brazil, where this species is an important source of meat and milk for humans, particularly in Northeast region, in which 93.7% of the goats are concentrated (Brasil 2009). The goat herd in Paraiba State ranks fifth of the national herd, and Cariri Occidental microregion represents the best market area of the country for its geographical location, higher density of goats and sheep on the continent and specially for having the best genetic material for both dairy and meat production.

In view of the importance of *C. abortus* in goat breeding and public health and the lack of data on its seroprevalence and factors that contribute to spread of infection in Brazil, this work aimed to estimate the prevalence and identify risk factors associated with anti-*C. abortus* antibodies in goats of the semiarid of Paraíba State, Northeast region of Brazil.

MATERIALS AND METHODS

Study area

The blood samples were collected from March to July 2009 at the municipality of Monteiro (7°53'S, 37°5'W), Cariri Ocidental microregion, semiarid region of the State of Paraíba, Northeast region of Brazil, which is characterized to be hot during the day and cold at night, with mean temperature of 22°C (71.6°F). The altitude is 599 above sea level. Monteiro excels in the goat milk production in Paraíba State and in Brazil, and has the major number of goats in the state with a total of 30,240 animals (Brasil 2009).

Study design and sampling

The study was designed as a cross-sectional study of randomly selected dairy goat flocks. Blood samples were collected from female goats that were ≥ 12 mo old. Adult females were used because in dairy goat flocks the females are kept in the flock, which would ensure identification of these animals for future activities.

A two-stage sample design was followed. Firstly, dairy goat flocks were randomly selected. The number of flocks to be sampled was determined considering the number of dairy goat flocks in the region (n=180, according to the data of the Center for Integrated Development of Goat Production, in Paraíba State), an expected flock prevalence of 50% (considering no a priori knowledge of the flock prevalence), and a 6% desired accuracy for a 95% level of confidence (Thrusfield 2007), resulting in 107 flocks to be sampled. Secondly, the sample size of goats to be selected was determined individually for each flock so as to detect the presence of the infection. Calculations were made in accordance with the formula commonly applied in veterinary epidemiological investigations (Thrusfield 2007):

$$n=[1-(1-p)^{\frac{1}{d}}] \times (N-\frac{d}{2}) + 1$$

where:
n = sample size;
p = probability of detection of at least one seropositive goat;
N = flock size;
d = number of seropositive goats in the flock.

The probability of detection of at least one seropositivity goat in a flock was determined at 95% (p=0.95), and the number of seropositivity goats in each flock (d) was calculated assuming within flock prevalence of 10% (Pereira et al. 2009).

Selection of goats to be sampled from each flock was based on a systematic random sampling, where goats were put in a crush pen and systematically selected. In situations where handling infrastructure was absent, true random sampling was difficult to attain. In such situations, animals were put in a kraal and "randomly" captured.

A total of 975 female goats in 110 flocks were randomly selected and examined for *C. abortus* infection.

Serum collection

A 10 ml blood sample from each animal was collected from jugular vein using vacutainer tubes. Samples were allowed to clot and sera were stored at -20°C until testing.

Serological procedure

Antibodies to *Chlamydophila abortus* were detected through the complement fixation test (CFT) (Donn et al. 1997), a method recommended by World Organization for Animal Health (OIE 2009). The reaction was conducted in microplates using test serum diluted from 1:16 to 1:512, antigen *C. abortus* S26/3 strain diluted 1:50 and the complement in the corresponding dilution to two fixating units of complement. After incubation at 37ºC for 30 min the hemolytic system was added to the microplates, incubated for 30 min and then centrifuged at 3000 rpm for 5 min. The results were read visually. Bovine serum with titer of 512 obtained from Institute Zooprofilattico Sperimentalle delle Venezie, Italy, and fetal bovine serum were used as positive and negative controls, respectively. The titer of antibody was defined as the reciprocal of the highest serum dilution that presented 50% complement fixation. Animals presenting titer 32 or higher were considered positive, and titer 16 were considered suspect.

Epidemiological data collection

A structured questionnaire focusing on risk factors for *C. abortus* infection was given to each farmer at the time of blood collection. Information was collected on a total of 18 flock-level factors that included: management system, main farm activity, flock size, predominant goat breed, presence of cattle, equine, swine and wildlife, availability of veterinary services, animal purchasing, mineral supplementation, vaccination against infectious diseases, lend buck for breeding, pasture rental, shearing pasture, use of disinfectants and use of maternity pens. History of abortions, infertility, stillbirths and birth of weak animals were also included.

Data analysis

Flocks that presented at least one seropositive animal were considered positive. Prevalence of positive flocks was estimated from the ratio of positive flocks to the total number of flocks investigated, with the exact binomial confidence interval of 95% (Thrusfield 2007), using the program EpiInfo version 6.04.

Risk factor analysis was performed in two steps: univariate and multivariate analysis. Univariate analysis was performed using the chi-square test or Fisher's exact test (Zar 1999), and those variables that presented $P \leq 0.20$ were used for multiple logistic regression. The multivariate analysis was then performed, using the stepwise forward method (Hosmer & Lemeshow 2000). The significance level in multivariate analysis was 0.05. The adjustment of the final model was checked using the Hosmer and Lemeshow test, and $P \geq 0.05$ was taken to indicate a satisfactory fit. The tests were performed using the SPSS for Windows software package, version 13.0.

RESULTS

Flock prevalence and frequency of seropositive animals

Fifty-five flocks out of 110 presented at least one seropositive animal with an overall prevalence of 50.0% (95% CI: 40.3%, 59.7%). On the animal level, 91 out of 975 dairy goats examined were seropositive with titers ≥ 32, resulting in a frequency of 9.3%. Four animals (4.4%) presented titer 16 and were considered suspect. Sixty-seven (73.6%) animals had titer 32 and 24 (26.4%) titer 64.

Risk factor analysis

For the risk factor analysis, one flock had at least one suspect animal and was excluded. Table 1 shows the distribution and respective numbers of examined risk factors. Goat rearing is the major activity, lend buck for breeding and history of abortions recorded a significant association with prevalence of positive flocks in the univariate analysis ($P \leq 0.20$). Final logistic regression model shown that lend buck for breeding (odds ratio = 2.35; P=0.041) and history of abortions (odds ratio =3.06; P=0.006) were associated with increased prevalence of positive flocks (Table 2). Fi-

Table 1. Relationships between risk factors and *Chlamydophila abortus* infection in flocks revealed in univariate analysis

Risk factor	Category	No. of flocks	No. of positive flocks (%)	P
Management system	Intensive	2	0 (0.0)	
	Semi-intensive	98	50 (51.0)	
	Extensive	9	5 (55.6)	0.343
Major activity: Goat rearing *	No	33	11 (33.3)	
	Yes	76	44 (57.9)	0.032
Flock size	< 25 goats	57	30 (52.6)	
	≥ 25 goats	52	25 (48.1)	0.777
Predominant goat breed	Pure	7	4 (57.1)	
	Crossed	102	51 (50.0)	1.000
Presence of cattle	No	35	18 (51.4)	
	Yes	74	37 (50.0)	1.000
Presence of equine	No	83	39 (47.0)	
	Yes	26	16 (61.5)	0.285
Presence of swine	No	78	40 (51.3)	
	Yes	31	15 (48.4)	0.952
Presence of wildlife	No	80	40 (50.0)	
	Yes	29	15 (51.7)	1.000
Veterinary services	No	103	53 (51.5)	
	Yes	6	2 (33.3)	0.438
Purchase animals	No	98	50 (51.0)	
from other farmers	Yes	11	5 (45.5)	0.974
Purchase animals	No	95	48 (50.5)	
from the market	Yes	14	7 (50.0)	1.000
Mineral supplementation	No	48	27 (56.3)	
	Yes	61	28 (45.9)	0.379
Vaccinate (infectious diseases)	No	21	12 (57.1)	
	Yes	88	43 (48.9)	0.661
Lend buck for breeding *	No	66	28 (42.4)	
	Yes	43	27 (62.8)	0.060
Pasture rental	No	104	52 (50.0)	
	Yes	5	3 (60.0)	1.000
Shearing pasture	No	96	48 (50.0)	
	Yes	13	7 (53.8)	1.000
Use of disinfectants	No	61	34 (55.7)	
	Yes	48	21 (43.8)	0.294
Maternity pens	No	100	49 (49.0)	
	Yes	9	6 (66.7)	0.489
History of abortions *	No	58	22 (37.9)	
	Yes	51	33 (64.7)	0.009
History of infertility	No	100	52 (52.0)	
	Yes	9	3 (33.3)	0.320
History of stillbirths	No	85	42 (49.4)	
	Yes	24	13 (54.2)	0.857
History of birth of weak animals	No	83	40 (48.2)	
	Yes	26	15 (57.7)	0.535

* All variables were statistically significant at P ≤ 0.2 and offered to the logistic regression model.

Table 2. Final logistic regression model for risk factors associated with *Chlamydophila abortus* infection in dairy goat flocks

Variables	β	S.E.	Odds ratio	95% CI	P
Lend buck for breeding	0.854	0.418	2.35	1.04, 5.33	0.041
History of abortions	1.117	0.408	3.06	1.37, 6.80	0.006

Hosmer and Lemeshow test: chi-square = 0.864; P = 0.649.

nal model had a good fit (Hosmer and Lemeshow test: χ^2 =0.864; P=0.649).

DISCUSSION

The frequency of seropositive animals found in the present study was 9.3%. Few works aiming to determine the frequency of antibodies against *Chlamydophila abortus* in goats in Brazil were performed (Piatti et al. 2006, Pereira et al. 2009). However, a random selected sample was not used. The use of convenience sampling in studies of the occurrence of infectious diseases is very common and allows the determination of important information; however, epidemiological inference should not be made based on this procedure in view of biases. Our work is the first study conduct with appropriate sampling design to determine the prevalence and risk factors of infection by *C. abortus* in goats in Brazil.

According to World Organization for Animal Health (OIE 2009), CFT can detect antibodies from vaccination or natural infection. In the State of Paraíba vaccination of goats against *C. abortus* infection is not practiced so that results obtained indicate the presence of infection. Sensitivity and specificity of the CFT have not been reported. However, CFT remains the recommended diagnostic technique for chlamydophilosis (OIE 2009).

According to epidemiological principles the sample was drawn by a simple random sampling and its size was determined so that it was possible to detect a seropositive animal in a flock with the probability of 95% (Thrusfield 2007). Among the 110 dairy goat flocks studied, 55 (50.0%; 95% CI: 40.3%, 59.7%) had at least one seropositive animal. This high frequency demonstrates that the agent is widespread in dairy goat flocks in the region. However, the definitive diagnosis depends on the detection of the agent, by cell cultivation or polymerase chain reaction (PCR). Pereira et al. (2009) in the State of Pernambuco, Northeast region of Brazil, evaluated goats and sheep by serological tests and reported a frequency ranging from 4.0% to 60.0% in 12 positive flocks for *C. abortus*. In 91.6% of the flocks at least one seropositive animal was found, indicating that the infection was widespread in the Northeast region of Brazil.

A low frequency of suspect animals was observed in this work (4.4%). These animals may have been infected, presenting titers of antibodies that would increase with time. The cross relation between *C. abortus* and *C. pecorum* can explain falso-positive results with low titers. Thus, titers lower than 32 must be considered unspecific for *C. abortus* (OIE 2009). In Brazil, there are no reports of goats and sheep infected by *C. pecorum* (Pinheiro Jr et al. 2010), however, this agent is endemic in ruminants in several countries, and can causes encephalitis, pneumonia, enteritis, polyarthritis, conjunctivitis and abortions in sheep, goats, cattle and pigs (Iowa State University 2005).

In the risk factor analysis, lend buck for breeding was identified as a risk factor for *C. abortus* infection in the dairy goat flocks. The sharing of bucks for breeding among owners is a very common practice in the region, and in most cases serological tests for detecting *C. abortus* infection are not performed. Thus, it is believed that this practice may be contributing for the spread of infection in dairy goat flocks in semiarid region of Paraíba State.

History of abortions in the flocks was also associated with prevalence of the infection. History of abortions should not be considered a risk factor, but a consequence of the *C. abortus* infection. This finding shown that *C. abortus* may be an important cause of abortions in goats in the region. Abortions in goats may have infectious or non-infectious etiology. Among non-infectious causes, dominate malnutrition, stress, mineral deficiencies as well as fetal anomalies. *C. abortus* infection is usually considered as the most important cause of caprine abortions (Chanton-Greutmann et al. 2002, Czopowicz et al. 2010) and it has been found to be the most common cause of abortions in goats in Hungary (Szeredi et al. 2006) and in USA (Moeller 2001).

CONCLUSIONS

The results indicate that the infection is widely disseminated in dairy goat flocks in the region, and control measures must be implemented mainly focusing on risk factor identified in this study, aiming to reduce the spread of infection and possible exposure of humans.

The investigation also suggests that *Chlamydophila abortus* may be an important cause of abortion in the area.

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