

Risk Factors Associated with Seroprevalence of *Chlamydia abortus* in Sheep Farms in Ceará, Brazil

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ABSTRACT

Background: *Chlamydia abortus* infections (Chlamydiosis) can cause reproductive problems in sheep, such as abortions and birth defects, leading to farm productivity loss. The symptoms, which are similar to other reproductive diseases, and the microbial pathogenesis make the clinical diagnosis difficult. *Chlamydia abortus* is a zoonotic pathogen, making it a public health issue because it can infect and induce abortions in humans. This study investigated anti-*C. abortus* antibody levels and infection risk factors in sheep in the State of Ceará, Brazil.

Materials, Methods & Results: Forty-three properties from 10 municipalities in 4 mesoregions in the State of Ceará, Brazil (Sertões, metropolitan Fortaleza, North Ceará and Northwest Ceará) with sheep, goats, cattle, and horses were visited. Five hundred and four serological samples from sheep were collected and tested for anti-*C. abortus* antibodies using an Enzyme-linked Immunosorbent Assay (ELISA) [IDEXX®, Australia] and all procedures were performed in accordance with the manufacturer's instructions at the Clinical Pathology Laboratory of EMBRAPA Goats and Sheep (Sobral, Brazil). Individual questionnaires were completed about sheep breeding practices and to identify possible *C. abortus* risks. Seropositive results were found in 18.45 % (93/504 individuals) of sheep, and 88.37 % (38/43 properties) of the herds had at least one seropositive animal. The number of seropositive individuals was significantly different between adults and ewes [$P < 0.01$; Odds Ratio (OR) = 0.510; 95% confidence interval (CI) = 0.306 - 0.850]. Logistic regression modeling identified a missing health certificate request for newly acquired animals as a chlamydiosis risk factor [$P = 0.038$; OR = 2.672; 95% CI = 1.058 - 6.749].

Discussion: The prevalence of anti-*C. abortus* antibodies in sheep in the State of Ceará emphasizes the importance of testing and tracking the disease spread among herds; these results were similar to studies in other areas of Brazil. Adult sheep that spend more time on the property may have a higher exposure risk because of increased reproductive activity. Misinformation and technical limitations can influence the proper handling of animals avoiding contagion through the correct use of techniques and recommendations. Disease transmission occurs through the digestive tract and between mother and fetus. Therefore, seropositive (infected) sheep may be related to the breeding system practices, such as allowing contact between sheep and other species on the property (goats, cattle, and horses) during breeding. Acquiring animals from external sources without sufficient health information can increase the transmission risk. Contaminated pastures, water, food, and air also increase transmission risk. The lack of technical and practical knowledge regarding disease prevention and control also contributes to disease transmission, resulting in reproductive losses due to high abortion rates. *Chlamydia abortus* has zoonotic potential and may infect humans without proper safety information. Therefore, future epidemiological studies are required for a better understanding of the primary risk factors for disease occurrence and spread among herds in the region. *Chlamydia abortus* infection is present in sheep in Ceará, Brazil. Chlamydiosis information programs should be adopted, sanitary measures implemented, and the epidemiological surveillance of sheep herds strengthened.

Keywords: *Chlamydia abortus*, epidemiology, sheep rearing, semiarid, serology.

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INTRODUCTION

Chlamydiosis is caused by a group of infectious pathogens from the family Chlamydiaceae, such as *Chlamydia suis*, *Chlamydia psittaci*, *Chlamydia abortus*, and *Chlamydia pecorum*, and affects both mammals and birds. It causes clinical manifestations such as conjunctivitis, arthritis, reproductive disease, and pneumonia and has an impact on both animal and public health [2,18].

The causative agent for ovine enzootic abortion is *C. abortus*, a gram-negative, obligatory intracellular bacteria, generally associated with reproductive problems [11]. It presents a characteristic biphasic development cycle, with two morphologically distinct forms [2]. The zoonotic implications of the bacteria are miscarriage in women and severe generalized infection with fetal loss [3,14].

Chlamydia abortus is prevalent in several countries and current research shows that its prevalence in sheep herds in Brazil ranges from 3.3 [20] to 21.5% [15] per animal and is up to 91.6% [13] in herds.

The clinical diagnosis of animals affected by chlamydiosis is considered complex. Complement fixation reactions, immunofluorescence tests, and enzyme-linked immunosorbent assays (ELISA) are frequently used to detect anti-*C. abortus*. Among these, ELISA has shown a sensitivity of up to 94.74% and a specificity of 95.6% [9,12,19].

Thus, based on the importance of sheep farming, the economic and zoonotic impact of chlamydiosis, and the lack of epidemiological information on *C. abortus* infection in sheep in the state of Ceará, the objectives of this study were to conduct a serological survey for *C. abortus* in sheep farms in Ceará, Brazil and to investigate the risk factors associated with its occurrence.

MATERIALS AND METHODS

Study area and sampling

The study was conducted in 4 mesoregions (Sertões, metropolitan Fortaleza, North Ceará and Northwest Ceará) in the state of Ceará, located in Northeastern Brazil. The Northeastern region accounts for 68.54% of the national sheep herds and 17.62% of these are present in the Ceará territory [6].

Forty-three sheep farmers/producers were chosen for the study using probabilistic sampling from a list of producers that was provided previously

by associations of breeders and municipal and state agriculture departments. The 4 mesoregions and 10 municipalities in the state of Ceará were selected owing to the significant animal densities in these areas.

The minimum number of samples to be collected was calculated according to a simple random sampling method [22] that considered a minimum expected prevalence of 21.5%, a sampling error of 2%, and a 95% confidence level. Given these parameters, a minimum sampling of 444 animals would be necessary, however 504 samples were used for this study. The selection of animals from each property occurred in a stratified manner, with 60% adult females (over 12 months of age), 35% young animals of both sexes (between 6 and 12 months of age), and all rams.

Ten to 12 ovine serological samples were obtained from each herd, based on the minimum number of animals to be examined in each herd. Blood samples were collected through jugular venipuncture using a vacuum tube without anticoagulant. These blood samples were then centrifuged at $3,000 \times g$ for 15 min to obtain serum, labelled, stored at -6°C , and transported in thermal boxes to EMBRAPA Goats and Sheep, Sobral, Ceará, where they were stored at -20°C until needed for further testing.

Serological diagnosis

The serum samples were tested using a commercial ELISA *C. abortus* antibody Test kit¹ with microtiter plates pre-impregnated with *C. abortus* antigen according to the manufacturer's recommendations. The results were obtained by reading the absorbance determined by a Thermo Scientific Fisher Multiskan FC^{®2} spectrophotometer with a wavelength of 450 nm to compare the optical density of the samples with that of the positive and negative controls.

Epidemiological questionnaire

Data were collected using an individual questionnaire for owners or those responsible for the herd. It consisted of questions formulated to obtain general information about the property, health aspects, reproductive practices, and composition of the herd. The questionnaires were filled out in loco by a group of trained technicians and scholarship holders from the Embrapa Goats and Sheep institute. The analyzed variables helped to evaluate the possible risk factors that could be associated with the presence of chlamydiosis in the evaluated herds.

Statistical analysis

The data obtained were analyzed using the Statistical Package for the Social Sciences (SPSS®)³ software for Windows version 21.0, where the magnitude of the association of risk factors was determined by the odds ratio (OR) and significance was determined when 95% of the confidence interval did not include 1. The analysis of the association between the groups was tested using the nonparametric Chi-square (χ^2) and Fisher's exact tests, with a statistical significance of 5% ($P < 0.05$) [24]. The variables submitted for univariate analysis that were related to chlamydiosis with a value of $P < 0.20$ were regrouped to perform logistic regression using the forward method [10].

RESULTS

Of the 504 serological samples tested, 93 (18.45%) had antibodies against *Chlamydia abortus*, and 88.37% (38/43) of the herd was positive for *C. abortus*. The presence of at least one seropositive animal was considered as a crucial factor to classify the property as the focus of infection. Based on the

analyses of the samples from the 4 mesoregions, there was no significant difference ($P > 0.05$) in the seropositive frequencies of the animals among the properties. It was observed that all ten participating municipalities had at least one property with a seropositive animal.

Seropositivity between adults (70/320; 21.88%) and young (23/184; 12.50%) ewes differed significantly from each other [$P = 0.009$, 95% CI = 0.305-0.850, OR = 0.5010]. However, there was no significant difference between males (18/102; 17.65%) and females (75/402; 18.66%). Male parent (12/54; 22.22%) and mother animals (58/266; 21.80%) presented similar seropositivity frequencies, as did those between young females (17/136; 12.50%) and young males [6/46; 12.50%; $P > 0.05$]. Results from different breeds showed 19.32% (957/295) seropositivity in pure-bred animals, 19.63% (32/163) in crossbred animals, and 8.70% (4/46) in mixed-breed sheep ($P > 0.05$).

It was observed that the levels of *C. abortus* infection were higher ($P < 0.05$) in sheep reared with cattle ($P = 0.019$), a situation also presented in sheep reared with equines ($P = 0.011$) [Table 1].

Table 1. Variables, obtained using univariate analysis, associated with *Chlamydia abortus* infection in sheep in the state of Ceará, Brazil.

Variable	Animals	% Positive	OR	CI 95%	P-value
Breeding system					
Extensive	24	3 (12.50)	1.615	0.472-5.533	0.594**
Semi-intensive	480	90 (18.75)			
Breed purpose					
Meat	385	69 (17.92)	0.864	0.515-1.451	0.581*
Mixed	119	24 (20.17)			
Goat breeding					
No	225	48 (21.33)	0.709	0.452-1.113	0.134*
Yes	279	45 (16.13)			
Cattle breeding					
No	111	12 (10.81)	2.142	1.121-4.091	0.019*
Yes	393	81 (20.61)			
Equine breeding					
No	134	15 (11.19)	2.119	1.172-3.832	0.011*
Yes	370	78 (21.08)			
Sheep and goat consortiation					
No	225	49 (21.78)	0.673	0.428-1.056	0.084*
Yes	279	44 (15.77)			
Worker training					
No	220	42 (19.09)	0.928	0.590-1.459	0.745*
Yes	284	51 (17.96)			
Technical assistance					
No	108	27 (25.00)	0.600	0.360-0.999	0.048*
Yes	396	66 (16.67)			

*Variables selected by the Chi-Square ($P \leq 0.20$). **Variables selected by Fisher's Exact Test ($P \leq 0.20$). OR= Odds Ratio. CI= Confidence interval.

Table 2. Variables obtained through univariate analysis, associated with *Chlamydia abortus* infection based on reproductive and sanitary management in sheep properties in the state of Ceará, Brazil.

Variable	Animals	% Positive	OR	CI 95%	P-value
Separation of sheep by age					
No	326	53 (16.26)	1.493	0.944-2.362	0.086*
Yes	178	40 (22.47)			
Separation of sheep by sex					
No	268	45 (16.79)	1.265	0.806-1.986	0.306*
Yes	236	48 (20.34)			
Sheep before giving birth					
No	136	21 (15.44)	1.332	0.783-2.267	0.289*
Yes	368	72 (19.57)			
Lambs with the mother					
No	48	5 (10.42)	2.057	0.792-5.343	0.131*
Yes	456	88 (19.30)			
Mortality at birth					
No	112	21 (18.75)	0.975	0.569-1.671	0.927*
Yes	392	72 (18.37)			
Reproductive practices					
Uncontrolled natural mount	176	34 (19.32)	1.092	0.683-1.744	0.714*
Natural controlled mount	328	59 (17.99)			
Breeder replacement					
No	23	2 (8.70)	2.450	0.564-10.637	0.280**
Yes	481	91 (18.92)			
Breeder's origin					
Own herd	36	4 (11.11)	1.879	0.648-5.448	0.370*
External herd	468	89 (19.02)			
Origin of the matrices					
Own herd	298	56 (18.79)	0.946	0.598-1.498	0.813*
External herd	206	37 (17.96)			
Vaccination of animals					
No	107	22 (20.56)	0.841	0.493-1.436	0.526*
Yes	397	71 (17.88)			
Lime at the animal installation					
No	287	53 (18.47)	0.998	0.633-1.572	0.992*
Yes	217	40 (18.43)			
Care of newly acquired animals					
No	220	38 (17.27)	1.150	0.728-1.817	0.548*
Yes	284	55 (19.37)			
Health certificate of newly acquired animals					
No	446	78 (17.49)	1.646	0.871-3.110	0.122*
Yes	58	15 (25.86)			
Separation of acquired animals					
No	278	53 (19.06)	0.913	0.580-1.438	0.694*
Yes	226	40 (17.70)			
Cleaning of facilities					
Does not clean	24	7 (29.17)	-	-	0.241*
Daily	71	17 (23.94)			
Monthly	301	49 (16.28)			
Annually	108	20 (18.52)			
Food reserve					
No	167	39 (23.35)	0.626	0.395-0.994	0.046*
Yes	337	54 (16.03)			

*Variables selected by the Chi-Square ($P \leq 0.20$); **Variables selected by Fisher's Exact Test ($P \leq 0.20$); OR= Odds Ratio. CI= Confidence interval.

Table 3. Multivariate logistic regression analysis of factors associated with the seroprevalence of *Chlamydia abortus* in sheep in the State of Ceará, Brazil.

Variable	B ^a	SE ^b	Wald	OR ^c	CI 95% ^d	P ^e
Failure to request a health certificate for newly acquired animals	0.983	0.473	4,325	2,672	1,058-6,749	0.038

Hosmers and Lemeshow Chi-square = 2.609; Freedom degrees = 8; P-value = 0.956. Ba= Logistic regression coefficient; SEb= Standard error; ORc= Odds ratio; CId= Confidence interval; Pe = P-value.

It was found that animals reared with no technical assistance (25.00%) demonstrated higher positivity for chlamydiosis, compared to those reared with this subsidy (16.67%; $P < 0.05$). Sheep raised without the practice of food reserve showed greater seropositivity than those that enjoyed this facility ($P < 0.05$) [Table 2].

In the final analysis of the logistic regression model, the variable lack of request for a health certificate for newly acquired animals was identified as a factor associated with the occurrence of chlamydiosis in sheep in the state of Ceará (Table 3). In the absence of this practice, 88.49% of the animals who did not have a health certificate tested positive compared to the 11.51% animals who were certified.

DISCUSSION

The observed prevalence of *Chlamydia abortus* was 18.45% (93/504) in animals and 88.37% (38/43) in sheep farms. This is considered high and confirms the presence of the bacteria in sheep herds in the regions studied. It partially explains the problems of abortion in small ruminants in the region. It is worth mentioning that, in a study carried out on goat farms in Ceará, abortion was considered the third major animal health problem [16]. However, studies on *C. abortus* in Brazil are still scarce, highlighting the absence of previous epidemiological studies on sheep chlamydiosis in the Ceará region.

The prevalence observed in this study reinforces the importance of acquiring epidemiological knowledge of the disease to identify the main factors and pathways of disease dissemination. In addition, obtaining information about the history of reproductive problems and obtaining results of serological analysis of the herd allows the identification of the main pathways of entry and permanence of the disease in the herd.

In the state of Alagoas, 21.53% of sheep were positive for *C. abortus* infection [15], 19.75% were positive in Paraíba [4], 8.20% in Piauí [7], and 8.13% in Pernambuco [13]. These results corroborate the

prevalence found in the present study. However, they differ due to the different strategies used in choosing the mesoregions, participating municipalities, and properties involved. Higher prevalence has been reported in Mexico (29.78%) [17], where the ELISA test was used to detect antibodies against *C. abortus* in sheep raised in coexistence with other species. It should be noted that vaccination against chlamydiosis is not carried out in sheep herds in Brazil.

The results from the present study show that adult animals (21.88%) are more prone to infection by *C. abortus* compared to young animals (12.50%; $P < 0.05$). This is due to the longer stay in the herd and greater chances of contact with the infectious agent. These findings do not differ from those obtained in Algeria, where a significant increase in prevalence was noted with the increase in age group [5]. Therefore, horizontal transmission is the main form of contamination in a sheep herd [8].

Sheep raised in the presence of cattle showed significant positivity for *C. abortus* (20.61%; $P < 0.05$). Sheep production in most farms in the present study was associated with the husbandry of other species of farm animals, including cattle, and the management practices adopted in such farms enabled contact between the animals as they shared facilities, water, and food sources. These conditions can favor the occurrence and transmission of diseases that affect cattle, including chlamydiosis. In the Paraná region, cows with a history of abortion showed 1.42% (44/3,102) positivity for *C. abortus* [21]. Therefore, it is believed that the transmission is amplified as a result of close and frequent contact between species.

It is worth mentioning that positive results can arise from cross-reactivity to *C. pecorum*, a species commonly found in ruminants [23]. Thus, the potential risk of inter-species transmission of the agent is emphasized, mainly in intercropping farms. Hence, it is important to inform and train technicians and producers about the risks of chlamydiosis.

In this research, it was noted that sheep when raised in the presence of equines showed greater

positivity for *C. abortus* ($P < 0.05$). These results are corroborated by the *C. abortus*-positive data obtained from sheep (29.7%) raised in the company of horses (1.32%), cattle (48%), and goats (12.5%) in Mexico [17]. Although bacteria in the Chlamydiaceae family have typical hosts, there is a great diversity of species that are affected by *C. abortus*, including cattle and horses [2].

Animals raised in the absence of variable technical assistance ($P > 0.05$) showed a higher seropositivity for *C. abortus* in comparison to those that were not. Therefore, the lack of specialized services can be favorable to the occurrence and spread of the infectious agent in herds. Consequently, the lack of adequate sanitary guidelines can facilitate the progress of the disease and negatively impact sheep farming. In addition, *C. abortus* presents risks to human health, especially to workers who are unaware of the disease and to pregnant women who deal with animals [14].

The significant correlation between food reserve absence ($P < 0.05$) and the occurrence of *C. abortus* in sheep found in this study highlights that sheep farming in Northeastern Brazil is still characterized by a deficit in nutritional advancements and, among other factors, the lack of technical assistance that may influence the reduced use of food storage techniques [1]. It is noteworthy that animals can acquire the infection by eating contaminated food.

From the variables selected for multiple analysis, the lack of request for a health certificate for newly acquired animals was the variable pointed out in the logistic regression as a risk factor for chlamydiosis in sheep.

The insertion of new animals in the herd, without previously obtaining necessary information about their health condition, is still a common practice among sheep farmers. The results obtained in this work pointed to the absence of a request for a health certificate for newly acquired animals as a factor associated with the presence of animals infected by *C. abortus*

in sheep herds in the Ceará state. The absence of this resource and the history of abortions associated with other reproductive disorders may act as a facilitator in the introduction of the disease and potentialize the spread of the infectious agent in the herd, in addition to favoring the entry of other pathogenic agents. Therefore, the acquisition of animals from safe sources and implementation of measures to prevent the entry of the pathogen in the herds, will help to minimize the risks of infection and the spread of *C. abortus*.

CONCLUSION

Infection with *Chlamydia abortus* is present in sheep in the state of Ceará and the lack of request for a health certificate for newly acquired animals is considered a factor associated with the occurrence of the disease. Therefore, it is important implement training for technicians and sheep producers on the correct management practices for adult animals, including pregnant and non-pregnant females, with regard to the occurrence of *C. abortus* in production animals and their zoonotic potential. It is also suggested that epidemiological surveillance by official institutions be intensified to combat the impact of the disease in sheep herds.

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