Epidemiological Evaluation of Reproductive Infectious Agents in Sheep in the Brazilian Semiarid

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Abstract: The aim of this work was to identify sheep herds with history of reproductive failures (abortions and perinatal mortality) associated to presence of *Chamydophila abortus, Brucella ovis* and *Leptospira* spp. infections in the semiarid region of Northeastern Brazil. Blood samples were collected from 476 animals from 72 herds in 14 counties of the Sertão mesoregion, state of Paraíba. For the serological diagnosis of *C. abortus*, *B. ovis* and *Leptospira* spp. infections, complement fixation test (CFT), agar gel immunodiffusion test (AGID) and microscopic agglutination test (MAT) were used, respectively. Herd-level prevalence (herds with at least one seropositive animal) of *C. abortus* was 52.8%, followed by *B. ovis* (33.3%) and *Leptospira* spp. (27.7%); concerning seropositivity in animals there was also a higher frequency of *C. abortus* (19.7%), followed by *B. ovis* (12.1%) and *Leptospira* spp. (7.6%) ($p < 0.05$). Herds with history of abortion (31.9%; 23/72) and perinatal mortality (54.2%; 39/72) had at least one sheep seropositive to one of the infectious agentes. For abortions, herd-level prevalence of *C. abortus* was 60.8%, followed by *B. ovis* (43.4%) and *Leptospira* spp. (30.4%). For perinatal mortality, herd-level prevalence was 64.1% for *C. abortus*, 38.4% for *B. ovis* and 33.3% for *Leptospira* spp. It is suggested that these agents may be important causes of reproductive failures in the semiarid region, and, thus, it is recommended that herd owners should be informed on reasonable sanitary measures related to animal health, and advised to perform diagnosis in cases of abortion and perinatal mortality.

Keywords: Reproduction diseases, small ruminants, epidemiology, control, Northeastern Brazil.

INTRODUCTION

Brazilian sheep husbandry has a prominent position due to its great potential for growth. In recent years, significant developments were observed in the national herd, which has more than 17 million heads of cattle, an increase of approximately 10% in the years 2005-2010 [1]. This expansion can be explained by some advantages provided by sheep husbandry such as there is no need for a very extensive area, lower food consumption, easy handling and great diversity of meat production and quality leather providing an alternative source of income. However, the productive potential of this activity in the national agribusiness remains small due to some issues related to management and integration of the primary sector of the productive chain.

In the semiarid region of Paraíba, Northeastern Brazil, the sheep industry constitutes one of the main economic activities, where the sale of live animals and/or skins is an additional source of funds to buy goods not produced in the farm. Approximately 57% of the total Brazilian sheep herd is in the Northeastern region, and 2.5% is in the state of Paraíba, distributed into approximately 19,744 farms [1].

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Sanitary deficiencies observed in the evolutionary process of Brazilian sheep husbandry need to be resolved. These deficiencies concern the damage caused by infectious diseases, mainly the infections due to *Brucella ovis*, *Leptospira* spp. and *Chlamydophila* sp., which are responsible for economic losses resulting from return to estrus, abortions and perinatal mortality [2, 3]. Thus, the aim of this study was to identify positive farms (outbreaks) with a com history of abortions and perinatal mortality associated to the presence of *C. abortus, B. ovis* and *Leptospira* spp. in wooless sheep in the semiarid region of Paraíba, using a planned sampling.

MATERIALS AND METHODS

The State of Paraíba is geographically divided into four mesoregions (Sertão, Borborema, Agreste Paraibano and Mata Paraibana) and 23 microregions. The main activity in the Sertão mesoregion is extensive livestock raising; the climate is predominantly semiarid, with annual average temperatures above 26°C, rainfall between 300 and 500 mm per year and the characteristic vegetation is the caatinga. In the present study, adult wooless sheep from the Sertão mesoregion were used.

A total of 476 animals from 72 farms in 14 counties of the Sertão mesoregion were used in the study (Figure **1**). These farms were selected based on

E-ISSN: 2310-0796/13

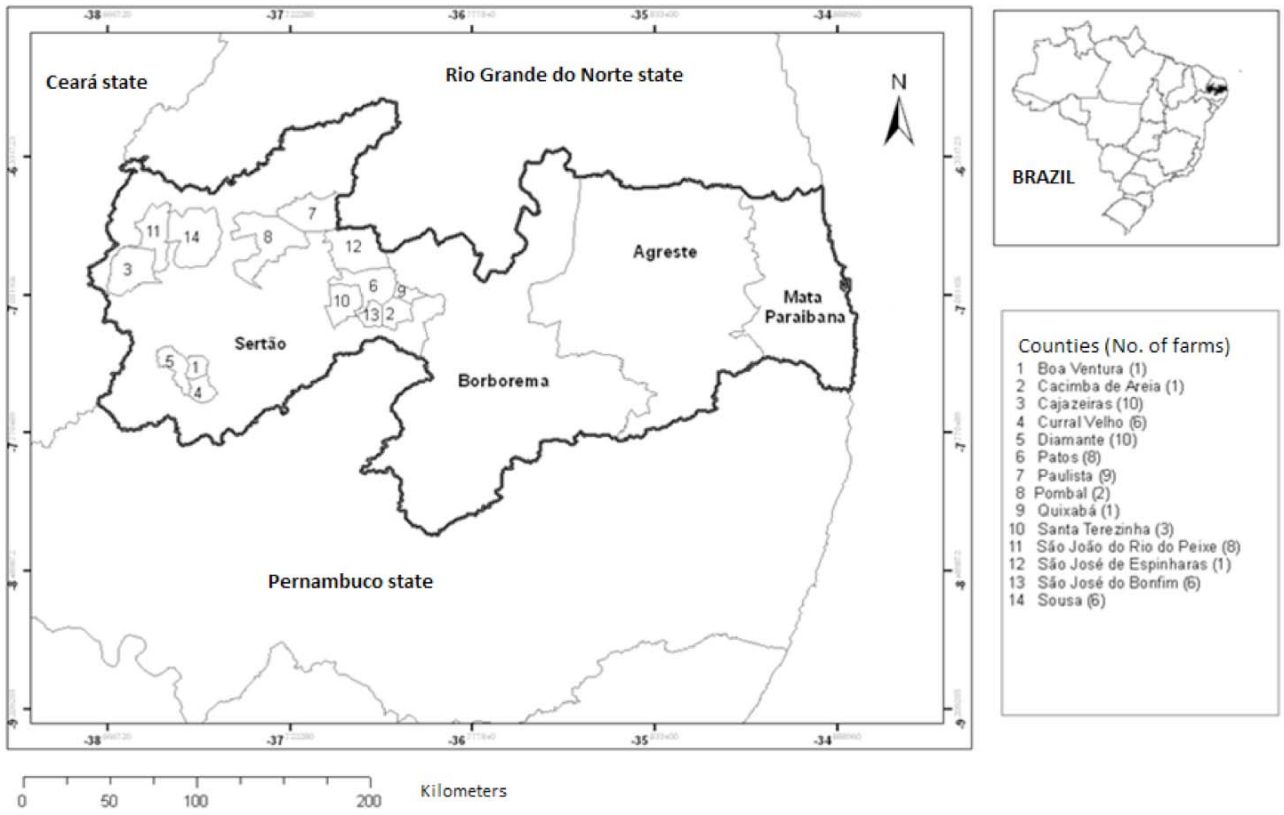

Figure 1: State of Paraíba with the counties and respective farms in the Sertão mesoregion of Paraíba.

previously planned samplings [5, 6, 11]. The sampling was designed to determine the prevalence of positive farms. The number of farms to be sampled was calculated with EpiInfo version 6.04 software [12], using the following parameters: expected prevalence of positive farms, confidence level of 99% and error of 10% [13], using the formula for simple random sampling. Then, the number of sheep to be selected for the detection of the infection was determined separately for each herd by using the formula for detection of disease [13].

Blood samples were collected from adult wooless sheep, in volumes of 8 ml, by jugular vein puncture using disposable needle and vacuum tube (without anticoagulant) with capacity of 8.5 mL. After draining, the sera were transferred to microtubes and frozen until the completion of serological tests.

The serological diagnosis of leptospirosis was made with Microscopic Agglutination Test (MAT) [14, 15], using a collection of live antigens that included serologically distinct types (serovars) Castellonis, Javanica, Tarassovi, Whitcombi, Australis, Autumnalis, Bataviae, Bratislava, Canicola, Copenhageni, Grippotyphosa, Hardjo (strains Hardjoprajitno and Hardjobovis), Hebdomadis, Pomona, Icterohaemorrhagiae, Sentot, Wolffi, Pyrogenes, Butembo, Cynopteri, Panama, Shermani, Andamana and Patoc. Sera were screened at a dilution of 1:100, and those with 50% or more agglutination were titrated by serial geometric dilutions of ratio equal to 2. The serum titer was the reciprocal of the highest dilution that showed a positive result. The antigens were examined using dark field microscopy, prior to the tests, in order to verify the mobility and presence of self-agglutination or contaminants.

For the diagnosis of *B. ovis* infection the agar gel immunodiffusion test (AGID) was performed with the use of kits produced by the Institute of Technology of Paraná (TECPAR)). The technique was performed according to the manufacturer's instructions, using lypopolysaccharides and proteins of *B. ovis*, sample Reo 198, as antigens.

For the detection of antibodies against *C. abortus* the Complement Fixation Test (CFT) was used [16]. The reaction was performed in microplates using test serum in 1:16 to 1:512 dilutions. The strain S26/3 of *C. abortus* at 1:50 dilution was used as antigen, and the complement at the dilution corresponding to two

complement fixation units. The antibody titer was considered as the reciprocal of the highest dilution of serum with 50% of complement fixation. Samples with titer equal to or higher than 32 were considered positive and with titer equal to 16 were considered suspected.

A farm was considered positive when at least one seropositive animal was detected. Chi-square test was used for comparison of the frequency of positive farms and seropositive animals for *C. abortus*, *B. ovis* and *Leptospira* spp. [17] with a significance level of 5%. Analyzes were performed with the BioEstat 5.03 software.

RESULTS AND DISCUSSION

According to the data obtained in this study (Table **1**), different prevalence rates of positive herds were observed for the agents investigated in the farms with history of reproductive problems, with at least one seropositive sheep, with emphasis to *C. abortus* (52.8%; 38/72), followed by *B. ovis* (33.3%; 24/72) and *Leptospira* spp. (27.7%; 20/72). Related to seropositivity in animals there was also a higher frequency for *C. abortus* (19.7%; 94/476), followed by *B. ovis* (12.1%; 58/476) and *Leptospira* spp. (7.60%, 37/476). Statistical significance was found in the comparison of the frequencies for *C. abortus* in relation to those for *B. ovis* and *Leptospira* spp. in both situations ($p < 0.05$).

Of the 14 counties visited, it is worth mentioning the countie of Paulista (Table **1**), where of the nine farms with animals that had reproductive failures the presence of *C. abortus* was detected in 77.8% (7/9), *B. ovis* in 55.5% (5/9) and *Leptospira* spp. in 33.3% (3/9) of them. Quantitative analysis of the animals indicated that 79 sheep were tested, with seropositivity for *C. abortus* of 22.8% (18/79), for *B. ovis* of 27.85% (22/79) and *Leptospira* spp. of 11.4% (9/79). The Countie of Paulista is located in the microregion of Pombal, an important area of breeding of wooless sheep, with intense commercialization of animals by farmers, which facilitates the spread of infectious agents.

Table **2** shows that of the 72 farms investigated, in 23 (31.9%) and 39 (54.2%) abortions and perinatal mortality, respectively, were reported, and all farms that reported such reproductive problems had at least one sheep seropositive for one of the agents. Regarding abortions, the study considered the farms with

Table 1: Prevalence of Positive Herds and Seropositive Wooless Sheep to *Brucella ovis*, *Chlamydophila abortus* and *Leptospira* spp. Infections in the Semiarid Region of Paraíba by Countie, from July 2010 to July 2011

Countie	Prevalence of positive herds Prevalence by animals							
	Total	No. of positive herds (%)			Total	No. of seropositive animals (%)		
		C. abortus	*B. ovis*	*Leptospira* spp.		*C. abortus*	*B. ovis*	*Leptospira* spp.
Boa Ventura	1	0 (0.0)	1 (100)	0 (0.0)	9	0 (0)	3 (33.3)	0 (0)
Cacimba de Areia	1	0 (0.0)	0 (0.0)	0 (0.0)	5	0 (0)	0 (0.0)	0 (0)
Cajazeiras	10	5 (50.0)	3 (30.0)	5 (50.0)	42	12 (28.6)	4 (9.5)	12 (28.6)
Curral Velho	6	3 (50.0)	0 (0.0)	0 (0.0)	48	6 (12.5)	0 (0.0)	0 (0.0)
Diamante	10	7 (70.0)	6 (60.0)	0 (0.0)	80	22 (2.7)	12 (15.0)	0 (0.0)
Patos	8	4 (50.0)	1 (12.5)	2 (25.0)	43	9 (20.9)	2 (4,6)	4 (9.3)
Paulista	9	7 (77.8)	5 (55.5)	3 (33.3)	79	18 (22.8)	22 (27.8)	9 (11.4)
Pombal	2	1 (50.0)	0 (0.0)	0 (0.0)	18	1 (5.5)	0 (0.0)	0 (0.0)
Quixaba	1	1 (100)	1 (100)	1 (100)	5	5 (100)	2 (40.0)	1 (20.0)
Santa Terezinha	3	1 (33.3)	0 (0.0)	2 (66.6)	17	4 (23.5)	0 (0.0)	2 (11.7)
São João do Rio do Peixe	8	2 (25.0)	2 (25.0)	2 (25.0)	48	3 (6.25)	5 (10.4)	3 (6.25)
São José de Espinharas	1	1 (100)	1 (100)	1 (100)	3	2 (66.6)	2 (66.6)	1 (33.3)
São José do Bonfim	6	3 (50.0)	1 (16.6)	2 (33.3)	39	5 (12.8)	1 (2.5)	2 (5.1)
Sousa	6	3 (50.0)	3 (50.0)	2 (33.3)	40	7 (17.5)	5 (12.5)	3 (7.5)
Total	72	38 (52.8)	24 (33.3)	20 (27.7)	476	94 (19.7)	58 (12.18)	37 (7.7)

Table 2: Prevalence of Positive Herds to *Brucella ovis*, *Chlamydophila abortus* and *Leptospira* spp. Infections According to Presence of Abortion and Perinatal Mortality in the Semiarid Region of Paraíba, from July 2010 to July 2011

Reproductive failure	Total number of herds (%)	Frequency of positive herds		
		C. abortus (%)	*B. ovis* (%)	*Leptospira* spp. (%)
Abortion	23/72 (31.9)	14/23 (60.8)	10/23 (43.4)	7/23 (30.4)
Perinatal mortality	39/72 (54.2)	25/39 (64.1)	15/39 (38.4)	13/39 (33.3)

notification of cases (23 farms) by the owner. In these farms, the prevalence of positive farms was higher for *C. abortus* (60.8%; 14/23), followed by *B. ovis* (43.4%; 10/23) and *Leptospira* spp. (30.4%; 7/23).

Perinatal mortality in sheep concerns the deaths occurring between 60 days of gestation until 28 days after delivery [2]. The farms that had at least one case of perinatal mortality such as pups born dead, death within 24 hours and death after weaning were considered. In the farms with identification of perinatal mortality, the frequencies of positive farms were 64.1% (25/39) for *C. abortus*, 38.4% (15/39) for *B. ovis* and 33.3% (13/39) for *Leptospira* spp. (Table **2**). The existence of dead pups reported by the farmers may lead to confusion in distinguishing this event from abortion, as observed by Leal *et al*. [18], who affirmed that farmers have difficulty in correctly diagnosis abortions, which are often confused with perinatal mortality.

The occurrence of *C. abortus* in herds of small ruminants has already been confirmed in some Brazilian states [7-11]. The association of abortions in sheep herds in Brazil to the presence of *C. abortus* was not confirmed, and it was found to be involved with other reproductive disorders, including the birth of dead pups [8, 19]. This fact demonstrates that the agent can be a major cause of the birth of dead pups in the region. Therefore, the identification of farms with history of abortions and other reproductive disorders becomes a critical element in the monitoring of herds and active control of the cases, and consequent identification of the involvement of *C. abortus* in damage to livestock in the region.

The frequencies of 43.4% and 38.4% of *B. ovis* in farms with history of abortion and perinatal mortality, respectively, demonstrates a possible role of the agent in reproductive disorders, causing losses to farmers that can often go unnoticed. These findings differ from those obtained by Marinho *et al*. [20], who did not report the presence of *B. ovis* and its association with reproductive problems. A noteworthy fact concerns the diagnostic techniques used and the possible cross-reactions between agents, interfering in the results. Two risk factors mentioned by Clementino *et al*. [4] and Santos *et al*. [5], purchase of animals and annual sanitation of the facilities, associated with inadequate management of the herd and poor facilities may favor the spread of *B. ovis* infection. The introduction of animals in the farms, by means of purchase or loans, without proper sanitary control allows dissemination of the agent. These practices are quite common among rural farmers in the Brazilian semiarid region.

Surveys with sheep in the semiarid region of Northeastern Brazil showed the presence of antibodies against *Leptospira* spp., with the predominance of the serovar Autumnalis, in which most of the positive animals did not show clinical signs of the disease [6, 21]. The frequencies for *Leptospira* spp. found in farms with perinatal mortality and abortion were 33.3% and 30.4%, respectively. According to Alves *et al*. [6], under the conditions of the Brazilian semiarid region, herd size (> 48 animals) and the participation of animals in exhibitions have been identified as risk factors for infection. Participation in exhibitions and herd size reflect the health conditions of the herd. This is emphasized by Faine [22] who affirmed that herd size is a critical factor for *Leptospira* spp. infection because animals in these agglomerations are more exposed to fomites, urine, vaginal secretions and abortion from infected animals.

CONCLUSIONS

It is concluded that in all farms with history of abortion and perinatal mortality there was at least one animal seropositive for *C. abortus*, *B. ovis* and *Leptospira* spp., as well as a higher frequency of positive herds and seropositve animals for *C. abortus* compared to *B. ovis* and *Leptospira* spp., suggesting that these agents may be an important cause of reproductive disorders in the semiarid region. It is suggested that efforts be concentrated in health education activities with farmers regarding prevention

and control of these infections, as well as direct diagnosis in cases of abortion and perinatal mortality.

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Received on 07-10-2013 Accepted on 22-10-2013 Published on 30-11-2013

DOI: http://dx.doi.org/10.12970/2310-0796.2013.01.01.3