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Detection of antibodies against *Chlamydophila abortus* in Costa Rican sheep flocks

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Abstract
A total of 359 sheep samples from 15 flocks were analyzed for the presence of antibodies against *Chlamydophila abortus* using a commercial Enzyme linked Immunosorbent Assay (ELISA). Antibodies were detected in 19 (5.29%) sheep from 12 (80%) flocks. Seropositive animals were found in all analyzed regions (Central, Chorotega, Atlantic Huetar, North Huetar and Central Pacific) determining prevalence between 0.28% and 4.4%, and intra-flock positivity between 3.7% and 25.0%. The survey revealed two risk factors associated with seropositivity; introducing animals (males and females), embryos, or semen from other farms or from abroad without any sanitary certification, and flocks not having quarantine areas or separated boxes for diseased animals. No clinical signs of disease were observed in positive seroreactors. *C. abortus* seems to be present in Costa Rica in a very low prevalence in sheep flocks. Further studies, to isolate the bacteria are required. Finally, implementation of control measures to prevent the spread of *C. abortus* is recommended.
Keywords: Abortion, Ovine, Serology, Tropics, Zoonosis.

Introduction

Ovine chlamydiosis, also known as ovine enzootic abortion (OEA) or enzootic abortion of ewes (EAE), is a zoonotic ovine disease caused by obligate intracellular gram-negative bacteria belonging to the family *Chlamydiaceae*. OEA is caused by *Chlamydia abortus* (formerly *C. psittaci* immunotype 1) which was identified as the most serious reproductive wastage agent in mammals and the major cause of reproductive loss in small ruminants worldwide (Aitken and Longbottom, 2007). *C. abortus* causes abortions in the last three weeks of pregnancy (Shewen, 1980). The infection is asymptomatic in some animals, showing no specific premonitory signs of the impending abortion, although some behavioral changes or vaginal discharge may be observed in some animals before the date of lambing. Infected ewes can also give birth to healthy lambs and it is not uncommon to observe delivery of a dead, weak or healthy lamb (Aitken and Longbottom, 2007).

Chlamydial infection can be diagnosed by identifying the organisms or their antigens in swabs, biopsies, secretions and tissue (blood, ocular discharges, placenta and fetal tissues) (Polkinghorne *et al*., 2009). The identification of extracellular infectious elementary bodies (EB) in smears can be carried out with Machiavelli, Giemsa, and modified Ziehl-Neelsen staining to differentiate them from *Brucella* spp. (Sachse *et al*., 2009).

Serological diagnosis is recommended to be carried out with paired sera. Serological assays include complement fixation (CF) test, enzyme linked-immunosorbent assay (ELISA) and micro-immuno-fluorescence assay. Despite the lack of species specificity, ELISA gives results with higher sensitivity than CF test and is widely used to test experimental and field samples (Anderson *et al*., 1995; Donn *et al*., 1997).

The presence of *C. psittaci* has been determined by polymerase chain reaction (PCR) assay in captive psittacine birds and in one feral pigeon from a public park in Costa Rica, (Dolz *et al*., 2013; Sheleby-Elías *et al*., 2013), while the presence of *C. abortus* was determined recently by ELISA and PCR in dairy cattle (Horigan, 2009; Fonseca, 2013). The presence of this agent in sheep flocks had not been studied to date. The aim of this study was to investigate the presence of antibodies against *C. abortus* in Costa Rica, using serological testing.

Materials and Methods

Studied population

Most of the selected sheep flocks were commercial flocks (87%), to produce tropical hair breed lambs (100%), and these animals were generally maintained intensively (93%). The sampled sheep breeds were Dorper, Pellybuey, Katahdin, Blackbelly, Texel, Suffolk, Santa Ines and their mixes.

Sample size

The sample size was calculated with an estimated population of 25,000 animals distributed in 138 sheep

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flocks in Costa Rica (1.0% overall expected prevalence, 95.0% confidence level), yielding a total of 300 samples to analyze. A total of 359 animals from 15 farms were tested. Within each flock, the number of animals to be sampled was calculated to determine presence or absence of *C. abortus* based on a 10.0% expected prevalence inside each flock (Kemmerling *et al*., 2009: Lensko *et al*., 2011), 95.0% confidence level and using the formula described by Cannon and Roe (1982). The flocks were distributed as follows: Seven in the Central region (46.0%), two in the Chorotega region (13.5%), two in the Central Pacific region (13.5%), two in the North Huetar region (13.5%) and two in the Atlantic Huetar region (13.5%). The Brunca region was not analyzed, since it was not possible to find farms willing to participate in this study. However, less than 10% of animals were registered in this region (Fig. 1).

Sample collection and survey

Blood was sampled from the jugular vein. Tubes were transported using coolers for keeping the temperature between 5°C and 10°C. In the laboratory, the samples were centrifuged for 5 minutes at 10,000 x *g* and sera were frozen at -20°C until processing by ELISA. Immediately after sampling, a questionnaire was supplied to the farmers to get information about housing, lamb husbandry, flock management, and presence of clinical signs (respiratory distress related to pneumonia, cough, sneeze, forced abdominal breathing associated with dyspnea, mucopurulent nasal or vaginal discharge, abortions, arthritis, conjunctivitis and weakness).

Enzyme-linked Immunosorbent Assay (ELISA)

The IDScreen® *Chlamydophila abortus* Indirect Multispecies ELISA from IDVet® (Montpellier, France) was used. This assay reported a sensitivity of 100% and specificity of 99.7% (Pourquier *et al.*, 2007). Major Outer Membrane Protein (MOMP) of *C. abortus* was adsorbed to the microtiter plates. The assay was carried out following the instructions of the manufacturer. Serum samples were analyzed in single wells, positive and negative control sera in duplicates. To validate the assay, average of the optical densities (OD) of the positive controls, and difference between averages of OD of positive and negative control sera were verified, to fulfill the limits specified by the manufacturer. With the optical densities obtained from the different sera samples, Serum Positive Percentage (S/P) was calculated, with respect to the average of the positive control sera, using the following formula: S/P = (OD of sample x 100): Average OD of positive control. As recommended by the manufacturer, serum samples that yielded S/P percentages less than 50% were considered negative, samples with S/P values between 50-60% were scored as weak positive reactors, and sera with S/P values greater than 60% were considered positive.

Fig. 1. Location of the participating flocks with *C. abortus* seropositive sheep (black dots) and seronegative animals (white dots) within five regions of Costa Rica.

Statistical analysis

Frequencies of the general characteristics and management practices of the sheep flocks were calculated. To assess the relationship between *C. abortus* and management practices (such as stabling, restricted access to sheep pens, having quarantine areas, having exclusive pens for lambs, feeding mastitic milk to lambs, buying animals or semen without any sanitary control, loaning males between farms, and presence of clinical signs), the odds ratio (OR) were calculated using a mixed effects logistic regression, with the sheep flock as the random variable. Due to the small numbers of positive samples, only a univariable analysis was performed for each independent variable. The data were analyzed using SAS/STAT ver. 9.2 (SAS Institute Inc.).

Results and Discussion

From a total of 359 sheep serum samples analyzed by ELISA, 19 reacted positively. No clinical signs of disease were observed in positive seroreactors. Most of the sera (314, 87.5%) gave S/P values lower than 30%, only 26 (7.2%) sera yielded S/P values between 30 and 50%, while 5.3% of the sera produced S/P values greater than 50% and were considered either as weak positives (8 animals, 2.2%) or as positives (11 sheep, 3.1%). 80% (12/15) of the flocks contained seropositive animals, while intra-flock positivity ranged between 3.7% and 25%. All five regions had seroreactors to *C. abortus*, where seropositivity ranged between 0.28% and 2.78%. The Central region had the highest numbers of seropositive animals (Table 1).

The questionnaire revealed two management risk factors associated with chlamydial seropositivity: Buying animals (males and females), embryos, or semen from other farms without knowing the sanitary status of *C. abortus* (59.05% of studied flocks), and the lack of quarantine areas or separated boxes for diseased

Table 1. Number and percentage of animals tested in 15 sheep flocks and distribution of seropositive individuals according to flocks and regions.

Farm identification	*Region*	*Total animals in flock*	*Animals tested*	*Positive animals (%)*	*Breed*	*Flocks analyzed*	*Regional positivity (%)*	*Global positivity (%)*
7	Central	80	25	2 (8.0)	D, K	7	6.29	2.78
8	Central	103	25	1 (4.0)	D, K			
9	Central	136	26	2 (7.7)	K, B, P			
12	Central	100	25	1 (4.0)	Om			
13	Central	220	26	3 (11.5)	Om			
14	Central	300	28	0	Om			
15	Central	4	4	1 (25.0)	D, K			
5	Central pacific	500	27	1 (3.7)	Om	2	7.27	1.11
10	Central pacific	200	28	3 (10.7)	Om			
2	Chorotega	115	25	1 (4.0)	D, K, P	2	5.88	0.84
3	Chorotega	140	26	2 (7.7)	Om			
4	Atlantic huetar	30	20	0	K, P	2	2.12	0.28
11	Atlantic huetar	350	27	1 (3.7)	D, K, S			
1	North huetar	200	21	0	D, K, P	2	2.12	0.28
6	North huetar	131	26	1 (3.8)	D, K, T			
	Total	2609	359	19		15		5.29

D: Dorper; K: Katahdin; P: Pelibuey; S: Suffolk; T: Texel; B: Blackbelly; Om: Other mixed breeds.

animals in each flock (55.71% of studied flocks, Table 2).

This study was the first to detect chlamydial antibodies in sheep flocks in Costa Rica. Our study revealed a widespread and low overall seropositivity (5.29%) of *C. abortus* in sheep in Costa Rica, similar to that described in dairy cattle by Fonseca (2013). Levels of seroprevalence determined in the different regions (2.12%-7.27%) were similar to that obtained in other sheep and goat studies conducted in small European territories such as Sardinia, Italy or Vorarlberg, Austria (Masala *et al*., 2005; Blumer *et al*., 2012). However, our findings did not agree with data published in other Latin American countries, such as Mexico or Brazil, which reported higher levels of seroprevalence (Jiménez-Estrada *et al*., 2008; Pinheiro Junior *et al*., 2010). One reason could be that the sheep industry is just emerging in Costa Rica. Nevertheless, mobilization of animals from one herd to another with different husbandry conditions occurs without any control, testing or quarantine, which facilitates quick and easy spread of infections through direct contact with other infected domestic and/or wild animals (Qin *et al*., 2014). This would also help to explain how 80% of the examined flocks were seropositive, while intra-flock positivity ranged between 3.7% and 25%.

The results obtained in the univariable analysis revealed a higher risk exposure to *C. abortus* infection in open sheep flocks (OR= 1.461, CI: 1.178 to 1.811) and flocks without quarantine (OR= 2.261, CI: 0.992 to 4.717), similar to results obtained by Pinheiro Junior *et al.* (2010).

Table 2. Risk factors associated with *C. abortus* seropositivity in sheep flocks in Costa Rica.

Variable	*% Flocks*		*OR*	*CI (95%)*	
	Positive	*Negative*		*UL*	*LL*
Open flocks	59.05	40.95	1.461	1.178	1.811
No quarantine areas	55.71	44.29	2.261	0.992	4.717

OR: Odds ratio; UL: Upper limit; LL: Lower limit; CI: Confidence interval.

The major sources of infection are the placental membranes, dead fetuses, coats of live lambs born to infected mothers, and vaginal discharges. Thus, affected animals need to be identified and isolated as quickly as possible and all dead fetuses, placental membranes, and bedding should be carefully disposed of. Also, lambing pens must be cleaned and disinfected (Stuen and Longbottom, 2011). This is very important as the lack of quarantine areas for diseased animals was recognized as a risk factor in the present research. In addition, ovine chlamydiosis has been shown to be an important zoonotic agent, affecting pregnant women, even with indirect contact with infected sheep or goats, principally in rural areas, and especially when simple

sanitary rules were not correctly followed (Meijer *et al*., 2004).

We conclude that positive results obtained in this study, were due to presence of antibodies against *C. abortus* in the Costa Rican sheep flocks, although cross-reactions with antibodies against *Chlamydia pecorum* are possible. However, the use of MOMP antigens in ELISA provides species-specific serodiagnosis (Hoelzle *et al*., 2004). We recommend carrying out further studies in order to isolate the agent from maternal and fetal tissues, and to implement surveillance measures, including regular testing for *C. abortus* and detailed investigations of ovine abortions.

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Conflict of interest

There is no conflict of interest in this study.

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