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Article

Frequency and risk factors associated with the presence of C*hlamydia abortus* in flocks of sheep in Mexico

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Abstract:

This study aimed to evaluate the individual and flock serological frequency and detect the risk factors of *C. abortus* infection in seven Mexican sheep producing states. It was performed a multifactor, cross-sectional, and stratified study with an analysis of 5,321 serological samples from 323 flocks in 61 municipalities. Serology frequency was determined using a commercial ELISA kit. The risk factors associated with the disease were determined through surveys and statistical analyses with a squared Chi test and a 95% confidence interval. Of the 5,231 serum samples, 581 (10.92 %) had positive ELISA test results. The results, by state, of positive sera were: Tlaxcala 13.08 % (73/558); Sonora 12.45 % (102/819); Chihuahua 11.56 % (107/925); Hidalgo 11.34 % (97/855); Chiapas 10.15 % (60/591); Querétaro 9.69 % (79/815); Estado de México 7.09 % (63/758). The frequency of seropositive herds was 43.34 % (140/323). The results, when grouped by state, were the following: Hidalgo 67.39 % (31/46), Querétaro 67.18 % (43/64); Sonora 40.92 % (19/47); Tlaxcala 33.33 % (12/36); Chiapas 31.57 % (12/38); Estado de México 25.45 % (14/55), and Chihuahua 24.32 % (9/37). The main risk factors that favor the presence of ovine enzootic abortion are gestation, 37 to 48 mo of age, and an intensive production system. These serology studies identified the presence of ovine enzootic abortion in Mexico and some of the risk factors associated with this infection.

Key words: *Chlamydia abortus*, Ewes, Seroprevalence, Risk factors, Mexico.

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Introduction

The ovine enzootic abortion (OEA) or ovine chlamydiosis is an infectious disease caused by *Chlamydia abortus*, an obligate intracellular Gram-negative bacterium. This bacterium has an affinity for mucous membranes; therefore, after the placental invasion, it tends to cause ulceration of the endometrial epithelium, abortion, or birth of weak lambs[1-2].

Abortion cases are critical for animal husbandry since they contribute to economic losses due to lack of lambs and milk production loss. *C. abortus* has zoonotic potential; it causes conjunctivitis, pneumonia, and abortion in humans[3-4].

The abortions caused by OEA occur in the last third of gestation without clinical signs before the abortion; these cases prevail in areas were flocks are kept in overcrowded spaces during calving seasons[3]. The clinical signs observed in flocks include epididymitis, pneumonia, arthritis, and conjunctivitis[5-8].

In Mexico, several studies have been performed in small ruminants; in 1996, *Chlamydia psittaci* was isolated from subclinical enteric infections in sheep flocks from five Mexican states[9], and in 1997, the first isolation of goat abortion was reported[10]. In 2001, it was reported as a zoonotic disease in Mexico, as humans became infected with *Chlamydia* spp from goats[11].

However, the fact that the disease was considered exotic in Mexico until May 2016 was a factor in the spread of the disease in our country, due to the lack of diagnostic methods[12]. In Mexico, there are no thorough epidemiological studies in sheep populations; however, the OEA is estimated to be widespread in this domestic species and, as such, is causing damage to sheep breeding nationwide. Additionally, the disease will likely continue to be introduced in many of the Mexican Republic states due to the exchange of animals between producers and to contact with other infected species, such as cattle or goats[13,14]. This study aimed to evaluate the serological frequency and the risk factors of *C. abortus* infection in the main sheep production areas in Mexico.

Material and methods

A total of 5,321 serum samples were collected from ewes older than 6 mo of age from 323 flocks in 61 municipalities of seven Mexican states: Hidalgo, Tlaxcala, Querétaro, Chihuahua, Sonora, Chiapas, and Estado de México. These states were chosen based on their productivity and the sample availability in different flocks. Most of the animals were of Mexican origin; imported sheep came from Australia, New Zealand, and the United States of America.

This was a multifactor, cross-sectional, and stratified study; flocks were selected based on the facilities granted by producers. The number of sampled animals was determined with the Win Epinfo Ver 2.0 software, using the percentage estimation mode, for an estimated 5% frequency of infected sheep, a 5% error, and 95% confidence. To determine the risk factors and their association with the presence of *C. abortus*, flock owners were asked to answer two surveys. The first focused on general aspects and flock management, considering genetics, nutrition, animal health, reproduction, and facilities. The second survey collected information from the sampled ewes: age, number of calvings, and clinical and production history.

The serodiagnosis was determined using an indirect ELISA (Pourquier® ELISA Chlamydiosis, IDDEX Maine, EE. UU.), which employs a recombinant protein antigen of 80-90 kDa, specific for *C. abortus*, without a cross-reaction with *Chlamydia pecorum*[15].

The frequency values of *C. abortus* for infected individual sheep and flocks were evaluated and compared with a Chi squared test, considering the frequency and the confidence interval of the ewes and their lambs, and the test parameters in comparisons, using the Win Epinfo Ver 2.0 software. The values with a *P* value <0.05 were considered significant, with a 95% confidence interval[16].

The risk factors associated with infection were evaluated with previously validated surveys. The data obtained were analyzed to determine the risk factors present in the flocks, which could be associated with the presence and epidemiological behavior of *C. abortus* in the sheep population (Table 1).

Table 1: Frequency and risk factors associated with the presence of *C. abortus* seropositive sheep

Factor	Category	Seropositive animals	Frequency (%)	OR	95% CI
Productive stage	Pregnant ewe	132/538	24.53	3.47 *	1.22-9.59
	Lactating ewe	235/1901	12.36	1.39 *	0.59-3.17
	Ewe nursing its lamb	22/170	12.94	1.41 *	0.54-3.36
	Ewe finishing lactation	106/1367	7.75	0.69	0.19-1.87
	Non-pregnant and non-lactating ewes	65/961	6.76	0.61	0.10-2.13
	Pubescent ewes in non-reproductive stage	21/384	5.46	0.49	0.06-3.92
Age	6 to 11 mo	46/949	4.84	0.97	0.20-3.30
	12 to 24 mo	78/1264	6.17	1.92*	0.50-4.10
	25 to 36 mo	101/1017	9.93	2.10*	1.15-4.07
	37 to 48 mo	356/2091	17.02	4.12*	2.62-6.34
Origin	Born in the flock	460/3897	11.80	1.9	0.70-4.90
	Bought	116/1331	8.71	0.7	0.30-1.60
	No data	0/37	0.00	0	0

Flock type	Intensive	95/525	18.09	2.23*	0.70-6.40
	Semi-intensive	370/3155	11.72	1.46*	0.50-3.20
	Extensive	116/1536	7.55	0.41	0.20-1.10
Total		581/5321	10.91		

OR=odds ratio; CI= concordance index.

* Statistical difference (P<0.05) associated with the risk factor.

Results

Of the 5,231 serum samples, 581 (10.92 %) had positive ELISA test results for the detection of antibodies against *C. abortus*. The frequency of seropositive animals grouped by state was: 12.45 % (102/819) in Sonora; 10.15 % (60/591) in Chiapas; 67.18 % (43/64) in Querétaro; 24.32 % (9/37) in Chihuahua; 33.33 % (12/36) in Tlaxcala; 11.34 % (97/855) in Hidalgo; and 7.09 % (63/758) in Estado de México. Of the total 323 sampled flocks, 43.34 % (140/323) had at least one *C. abortus* seropositive ewe.

The frequency values per flock were: 40.42 % (19/47) in Sonora; 31.57 % (12/38) in Chiapas; 67.18 % (43/64) in Querétaro; 24.32 % (9/37) in Chihuahua; 33.33 % (12/36) in Tlaxcala; 67.39 % (31/46) in Hidalgo; and 25.45 % (14/55) in Estado de México.

Regarding the productive stage of the sampled animals, 24.53 % of the seropositive animals were pregnant; 12.36 % were seropositive lactating ewes; 12.94 % were ewes nursing their lambs; 7.75 % were ewes at the end of their lactation; 6.76 % were non-pregnant and non-lactating ewes, and 5.46 % were pubescent ewes in a non-reproductive stage. The productive stage was evaluated as a possible risk factor, and the study found that pregnant ewes were 3.5 times more likely to be seropositive to *C. abortus* (Table 1).

Regarding seropositivity and age, 17.02 % of the seropositive animals were between 37 and 48 mo of age, in contrast with the groups between 6 to 11 mo, the frequencies of seropositive animals were very low since only 46 of the 949 sampled animals were positive. Based on the obtained odds ratio (OR), animals between the ages of 37 and 48 mo were 4.12 times more likely of infection than any other age (Table 1).

Concerning animal origin, 11.8% were animals born in the same flock, and 8.71% were acquired from other places; after analyzing this variable, origin was not considered a risk factor (Table 1).

As for flock management, 18.09% (OR = 2.23; 95% CI: 0.70-6.40) of the studied flocks were from intensive production units, 11.72% (OR 1.46; 95% CI 0.50-3.20 were semi-intensive, and the remaining 7.55% (OR = 0.41; 95% CI 0.20-1.10) came from extensive flocks (Table 1).

Discussion

The OEA was considered an exotic disease in Mexico until May 2016, when it became a notifiable disease; this study establishes a frequency of 10.92 % and confirms the presence and spread of the disease in the main sheep production areas in the country. Estado de México is the only state with previous OEA prevalence studies[9,12]. In this study, the data for Estado de México show that 7.09 % (n= 758) of the sampled animals were positive, while previous studies[9,12] reported a prevalence of 40.64 % and 21.3 %, respectively. However, these authors worked in flocks with reproductive problems, while, in the present study, animals were sampled without considering their reproductive status. Moreover, the high prevalence reported by Escalante in 1996 could be because they used a soluble antigen for their ELISA, which differs from the one used in this study which had a sensitivity of 95.7% and a specificity of 100%, this antigen is specific for *C. abortus*, avoiding the possibility of cross-reaction with *C. pecorum* or some Gram-negative bacterium such as *Acinetobacter* spp.

The highest frequency of OEA (13.08 %) was found in Tlaxcala, in flocks under intensive and semi-intensive production. These frequency numbers coincide with those described by Aitken[4], who reported that, in intensive production systems, the prevalence of OEA and reproductive disorders are higher than in extensive production systems.

The most important risk factor that promotes the spread of the disease in flocks is the introduction of animals not previously certified as negative to *C. abortus*[17], which was determined in the high percentage of positive flocks in the main sheep producing states in Mexico. Since OEA was considered an exotic disease, there were no commercial tests available to diagnose it, so it was not possible to prevent the spread of chlamydia.

The countries of origin of the imported sheep are mainly Australia and New Zealand, which are free of *C. abortus*[4]. However, imported animals are not tested before their arrival and are in close contact with the native sheep. This implies that the introduction of animals from endemic countries, like the United States of America, could be one of the factors associated with the spread of the disease, since this control is not carried out. This is supported by the fact that some imported ewes had late-stage abortions during quarantine periods and shortly after being introduced into the flocks.

Countries that are mainly dedicated to sheep farming show the greatest amount of problems related to abortions induced by OEA, the contact of infected sheep with contaminated material is one of the ways in which the disease can be transmitted to other animal species[1,18]. A study performed in Iran[19] determined the risk factors in small ruminants with abortions, of the 300 aborted fetuses (183 goats and 117 ewes), 11 % were PCR positive to *C. abortus*, determining that animal handling is an important risk factor because being in contact with each other facilitates the transmission of the disease.

The production system was identified as an associated risk factor; however, the highest seropositive frequency (18.9 %) was found in flocks under intensive production systems, which, due to the nature of this system, facilitates the spread of OEA and other diseases[20]. Moreover, this study confirms that sheep farming under extensive production protects against OEA (OR= 0.41; 95% CI: 0.2-1.1), which confirms that the spread of the disease is accelerated by animal overcrowding, as the contact between healthy and infected sheep increases in intensive and semi-intensive flocks[17,19,21,].

The relationship between the origin and the frequency of *C. abortus* seropositive ewes was considered an associated risk factor. Therefore, it is more likely that animals from production units in other states or countries, gathered upon arrival at distribution centers, are more easily infected due to their confining in facilities difficult to clean and disinfect[18].

Different studies support the fact that one of the main risks for the transmission of OEA is the place of origin of the animals, this allows to infer that in animals from farms in other states or even from other countries that are confined in distribution centers before arriving to their final destination, due to overcrowding and the lack of adequate means to clean and disinfect the facilities, the infection can spread more easily[17]. Regarding the animal origin; the only requirements that the producers consider before introducing a new sheep into their flock is the phenotype and that the animal looks clinically healthy, neglecting the diagnosis of the disease[1]. This study indicates that the bacterium has spread all over the country, but at different rates and proportions in the different states. However, when determining whether there was a relationship between the origin and the frequency of *C. abortus* seropositive ewes, it was not considered a risk factor.

This study identified that pregnant ewes are 3.4 times more likely to be infected. This could be because during gestation, ewes are immunosuppressed and, therefore, their nutritional demands increase, which is aggravated if their diet is inadequate. This is a result of the stress created by a deficient food source, which generates endogenous cortisol, a toxic substance for lymphocytes that increases immunosuppression[18].

The lack of strategies to separate infected ewes that are close to calving from the ones recently calved contributes enormously to infection because infected ewes shed high amounts of bacteria before, during, and after calving or abortion[1,22,23].

It was observed that as the age increases, the probabilities of exposure to the disease increase proportionally; therefore, the number of seropositive ewes increases. Ewes that have been in the flock for extensive periods are good mothers, but, with age, they resent the consequences of the number of births they carry and, therefore, their susceptibility to the disease increases[24,25]. Other factors may be involved in the spread of the disease, especially if proper biosecurity measures are not taken. For example, intensive production systems favor the contamination of pens, as there is a large accumulation of feces that cannot be recycled, therefore causing air, soil, and water pollution, which is related to the lack of equipment and the hygiene measures observed in the flocks examined in this study, and because the water is contaminated with feces and other organic materials[18,26].

The elevated frequencies of 43.34 % (140/323) in flocks were constant in the main sheep production areas in Mexico, which indicates that the OEA is widely spread all over the country, and with it, the consequences that have been reported by different authors[9-11,15], pointing to OEA as one of the main causes of abortion, which has a high economic impact in European, North American, and African countries[27], and due to the lack of the necessary diagnostic tools, the causes of its introduction to the country have not yet been determined, it is possible to suffer similar consequences to those suffered by the countries indicated above.

The spread of OEA does not only concern sheep production; previous studies have reported, through the diagnosis made by ELISA in dairy cattle with a history of abortion from eight Mexican states, a frequency of *C. abortus* positive animals of 14 % (145 /1,032)[14]; in bovines, the presence of OEA-positive animals is related to the presence of abortions and other reproductive problems[28].

Another study reported a 9.60 % frequency of *C. abortus* seropositive animals in six goat flocks in Guanajuato; the bacterium was isolated in 26.98 % of the sampled goats[13]. In a study performed in goats with abortions in Querétaro, Veracruz, Puebla, Jalisco, and the Comarca Lagunera region, *C. abortus* was isolated in 23.1 % of the samples[29].

Conclusions and implications

It was concluded that the positive serology frequency observed in the flocks of main sheep producing areas in Mexico, as well as the detection of risk factors associated with the presence and spread of the disease, evidence the spread of the ovine enzootic abortion in Mexico.

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