SHORT NOTE [NOTA CORTA]

SEROEPIDEMIOLOGY OF GOAT PARATUBERCULOSIS IN FIVE MUNICIPALITIES OF CENTRAL VERACRUZ, MEXICO

[SEROEPIDEMIOLOGÍA DE LA PARATUBERCULOSIS CAPRINA EN CINCO MUNICIPIOS DEL CENTRO DE VERACRUZ, MÉXICO]

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SUMMARY

Seroprevalence of goat paratuberculosis and risk factors were determined in flocks from five municipalities in the center of the state of Veracruz, Mexico, by a cross-sectional study using a stratified multistage approach. Sample size was calculated with the program Win Episcope Version 2.0 using the mode "estimate percentages" for 50 % seroprevalence, 5 % error and 95 % confidence, resulting in 182 animals and six animals per flock. According to the tables by Cannon and Roe, a sample size of 26 flocks was obtained, of which six flocks were sampled in the municipality of Tlacolulan and five flocks in each of the remaining four municipalities (Chiconquiaco, Yecuatla, Coacoatzintla and Coatepec). Identification of antibodies against *Mycobacterium avium* ssp. *paratuberculosis* was made by indirect ELISA. Seroprevalence was determined with the program VassarStat® for calculating ratios, and the risk factors by odds ratio. Overall seroprevalence was 0.6 % ($_{95\ \%}$ CI: 0.03 - 3.5). Reactors were only observed in Coatepec. Seroprevalence by municipality was 20 % ($_{95\ \%}$ CI: 1.0 - 70.12) and by flock 3.85 % ($_{95\ \%}$ CI: 0.2 - 21.59). There were no risk or protective factors detected. In conclusion, goat paratuberculosis is scarcely distributed in flocks from central Veracruz.

Key words: Seroprevalence; risk factors; distribution; goats; paratuberculosis.

RESUMEN

Se determinó la seroprevalencia de paratuberculosis caprina y factores de riesgo en rebaños caprinos de cinco municipios del centro del estado de Veracruz, México, mediante un estudio transversal polietápico estratificado. El tamaño de muestra se calculó con el programa Win Episcope Ver. 2.0 en modalidad "estimar porcentajes" para seroprevalencia 50 %, error 5% y confianza 95%, obteniéndose 182 animales y seis animales por rebaño. De acuerdo con las tablas de Cannon y Roe, se obtuvo un tamaño de muestra de 26 rebaños, de los cuales seis se muestrearon en el municipio de Tlacolulan y cinco en cada uno de los cuatro municipios restantes (Chiconquiaco, Yecuatla, Coacoatzintla y Coatepec). La identificación de anticuerpos contra *Mycobacterium avium* ssp. *paratuberculosis* fue mediante ELISA indirecta. La seroprevalencia se determinó con el programa VassarStat® para cálculo de proporciones y los factores de riesgo por razón de momios. La seroprevalencia general fue 0.6 % (IC $_{95\ \%}$: 0.03 - 3.5). Solo se observaron reactores en Coatepec. La seroprevalencia por municipio fue 20 % (IC $_{95\ \%}$: 1.0 - 70.12) y por rebaño 3.85 % (IC $_{95\ \%}$: 0.2 - 21.59). No se encontraron factores de riesgo ni protectores. En conclusión, la paratuberculosis caprina tiene baja distribución en rebaños del centro de Veracruz.

Palabras clave: Seroprevalencia; factores de riesgo; distribución; cabras; paratuberculosis.

INTRODUCTION

Paratuberculosis is a chronic disease that affects many animal species, particularly ruminants and within these goats, causing granulomatous enteritis. The causative agent is an alcohol-acid-resistant bacillus belonging to the genus *Mycobacterium* (Carter and Wise, 2004). Paratuberculosis is a disease that causes progressive

emaciation and has no specific clinical signs, only diarrhea is rarely seen in terminal stages, which makes diagnosis based on clinical recognition almost impossible (OIE, 2008). In some countries this disease has become the leading cause of death and/or waste, because it causes a considerable drop in milk production; hence, it is important in dairy goats (Soberón, 2011).

Mycobacterium avium ssp. *paratuberculosis* (MAP) is the causative agent of paratuberculosis or Johne's disease. This bacillus is resistant to many antibiotics due to its intracellular location and the presence of mycolic acids in its cell wall, so no treatment is effective against it. It also remains viable in the environment for weeks or even months, and can withstand pasteurization, in particular if it is poorly performed (OIE, 2008). Transmission occurs mainly by the fecal-oral route (Jaimes *et al*., 2008), and animals are infected within the first weeks of age due to ingestion of milk contaminated with feces or even by the bacteria passed from the lactating does to the kids (OIE, 2008). The bacteria may remain dormant in the mesenteric lymph for months or years, and clinical manifestations may be observed until adulthood. In the infected animal, stress can contribute to activate infection in the lymph nodes, triggering the release of MAP in feces and the later manifestation of diarrhea (Lambeth *et al*., 2004; Soberón, 2011). In addition, MAP has been associated with Crohn's disease in humans, and although it remains controversial, it could be considered as an important zoonosis (Acha and Szyfres, 2003). Because the isolation of this bacteria is delayed and complicated since its culture takes up to 12 weeks, other tools have been used for diagnosis, such as serological tests (ELISA), histopathology and molecular techniques (PCR) (Soberón, 2011).

The greatest variety of MAP strains has been observed in the USA, but there are many others in Australia, New Zealand and Europe (Whittington and Sergeant, 2001). In Mexico, Chávez *et al.* (2004) identified the strains of MAP by genetic polymorphism in a flock with serological and bacteriological evidence of paratuberculosis, and found that strain C1 was the most common as in other countries. Strain C1 is not specific to goats, and the lack of an official requirement to import paratuberculosis-free animals into Mexico represents a risk of entry of subclinically infected animals to local flocks. In Mexico, no extensive seroepidemiological studies have been conducted in goats, but it is estimated that the disease has spread in the country by importing breeding stock from the USA (Soberón, 2011). In the states of Querétaro and Morelos, which had clinical and serological evidence of paratuberculosis, MAP strain C1 was identified by PCR in milk from goats and was considered as the strain with the highest distribution (Favila-Humara *et al*., 2007).

In Mexico there have been efforts to prevent transmission of the disease. For instance, in 2009 a workshop on strategic planning for care of paratuberculosis in cattle, sheep and goats was conducted (CONASA, 2009); however, it was concluded that more studies are needed to determine the prevalence of the disease throughout the country, in order to give more attention to the states or municipalities with greater goat inventory, and to those where goat production is the main economic activity of the households. In Veracruz State, goat inventory is comparatively low, and 90 % of goat activities are developed in 14 municipalities in the central area, which also correspond mainly to marginalized municipalities (INAFED, 2005). As a consequence, there is no information available neither on the presence of MAP in goat flocks nor on the productive and economic impact of the disease. Hence, the objective of this study was to determine the seroprevalence of goat paratuberculosis and associated risk factors in five municipalities of central Veracruz, Mexico, during the year 2010.

MATERIALS AND METHODS

Location

The study was conducted from February to June 2010 in the municipalities of Coatepec, Chiconquiaco, Yecuatla, Coacoatzintla and Tlacolulan, located in the central region of Veracruz, Mexico, at latitude 19º 27” and 19°65” N and longitude 96°47” and 97°00” W. The elevation varies between 885 and 2,400 m above sea level and the climate is humid temperate, with temperatures ranging between 12 and 25 °C.

Study design

This was a stratified cross-sectional multistage study and flocks were randomly selected by clusters. The number of animals was calculated using the program Win Episcope Version 2.0 (Thrusfield *et al*., 2001) under the "estimate percentages" mode, for an estimated seroprevalence of 50 %, error 5 % and confidence 95 %. A total sample size of 182 animals with a sample fraction of six animals per flock was estimated. The number of flocks to be sampled was calculated according to the table by Cannon and Roe (1982). Female goats older than 3 months of age and all bucks were sampled.

Sampling

Sampling was performed by puncturing the jugular vein with Vacutainer® type tubes that were transported at 4 °C to the Laboratory of Microbiology, School of Veterinary Medicine, University of Veracruz, where they were centrifuged to separate the

serum, which was then stored in Eppendorf® conical tubes at -20 °C until serological processing.

Parallel to the sampling, two questionnaires were applied for each flock. The first questionnaire collected information on the general management including aspects such as breeding, feeding, water sources, management of excreta and cleaning of facilities. The second questionnaire was applied for each animal sampled and included data such as age, breed, body condition, sex, place of origin and medical background on the presence of diarrhea. Data from these questionnaires provided the variables that were used for further analysis.

Diagnosis

Commercial kits (IDEXX Laboratories, Inc., France) utilizing indirect ELISA procedures were used for the identification of IgG antibodies against MAP.

Data analysis

Seroprevalence and 95 % confidence intervals were calculated by Vassarstats® program. Association between variables was estimated by odds ratios (OR) in accordance with Thrusfield (2005).

RESULTS AND DISCUSSION

Overall seroprevalence

Overall seroprevalence of paratuberculosis in goats from the municipalities studied was 0.6 % ($_{95\ \%}$ CI: 0.03 - 3.5), which is lower than the 9.1 % found in Chile (Kruze *et al*., 2007), the 26 % reported in Spain (Stevenson *et al*., 2009), and the 24 % in dairy goats and 50 % in meat-producing goats from Argentina (Underwood and Carfagnini, 2005).

At the national level, flocks from the central Valley of Mexico showed a prevalence of 6.7 % (Chávez *et al*., 2004). In addition, in slaughterhouses Méndez *et al*. (2008) reported a prevalence of paratuberculosis lesions confirmed by histopathology and PCR ranging from 1.2 to 8.4 %. On the other hand, a descriptive analysis of cases received in a laboratory in Querétaro, reported no seropositive animals (Méndez *et al*., 2008), so there is a certain similarity with this study because values fall within the calculated confidence interval ($_{95\ \%}$ CI: 0.03 - 3.5 %).

Seroprevalence by municipality

In the five municipalities studied, only one seropositive animal was found, and it was from Coatepec (Table 1). This implies a seroprevalence of 20 % ($_{95\ \%}$ CI: 1.0 - 70.12) (Table 1). The survey showed that Coatepec has the greatest proportion of goats that have been introduced from other states. This result is in accordance with reports by Chávez *et al*. (2004) and Soberón (2011), who suggested that the introduction of MAP to goat herds in Mexico has been a consequence of importation of animals from infected areas. In regard to the only animal that tested seropositive in the flock and municipality, it came from the state of Guanajuato.

The flock in which the positive animal was detected has limited contact to other neighboring farms. This fact constrains the spreading of the disease. On the other hand, ELISA has high specificity (99.8 %) but rather low sensitivity (Cox *et al*., 1991), thus, the capability of the test to detect the antibodies against MAP may also have had an influence in this study.

Table 1. Seroprevalence of goat paratuberculosis by municipality in the central region of Veracruz, Mexico.

Municipality	Seroprevalence %	$_{95\ \%}$ CI	Odds ratio	OR $_{95\ \%}$ CI
Coatepec	100	5.46 - 100	1	0.5 - 2
Chiconquiaco	0	0 - 13.34	0	0
Yecuatla	0	0 - 12.70	0	0
Coacoatzintla	0	0 - 12.01	0	0
Tlacolulan	0	0 - 11.17	0	0
Total	20	1.0 - 70.12		

$_{95\ \%}$CI = $_{95\ \%}$ confidence interval for the seroprevalence; OR $_{95\ \%}$CI = $_{95\ \%}$ confidence interval for the odds ratio.

Seroprevalence by flock

Seroprevalence by flock was 3.85 % (1/26; $_{95\ \%}$CI: 0.2 - 21.59). Again, only one animal from the 26 flocks included in the study was seropositive. This particular flock had two bucks that came from outside the state of Veracruz, and one of them turned out to be seropositive, which was brought from the state of Guanajuato. Favila-Humara *et al*. (2007) when working with goat flocks in the states of Querétaro and Morelos in central Mexico, confirmed by PCR the presence of MAP in flocks with previous clinical

history and serological evidence; these authors pointed out to the purchase of undiagnosed infected animals as the possible source of infection for the animals that got infected, similar to what might be the case in the present study.

Seroprevalence by type of manure management

In the farm from which the seropositive animal came, animal manure was integrated into the fields as fertilizer (Table 2). Among farms, there were different manure management practices, such as manure distribution on pastures or compost preparation, among others. According to Díaz *et al.* (2005), the main source for paratuberculosis infection is the ingestion of the organism present in the fecal material. The use of feces as fertilizer for pastures may act as a reservoir for the germ. MAP may remain viable for weeks or months and the animals become infected by grazing in contaminated pastures. In this regard, and perhaps what has helped to contain the infection within the affected farm, is that the seropositive animal may still have the bacteria sequestered within any of its lymph nodes (Soberón, 2011).

Table 2. Seroprevalence of goat paratuberculosis in flocks by municipality and by practice of manure management and cleaning of facilities, in the central region of Veracruz, Mexico.

	Herds	Seroprevalence %	$_{95\ \%}$CI	Odds ratio	OR $_{95\ \%}$CI
Municipality					
Coatepec	5	20	0 - 70.12	56.61	0.080 - 3.99
Chiconquiaco	5	0	0 - 53.71	0	0
Yecuatla	5	0	0 - 53.72	0	0
Coacoatzintla	5	0	0 - 53.73	0	0
Tlacolulan	6	0	0 - 48.32	0	0
Manure management					
On pasture	26	3.85	2 - 21.59	0.006	0 - 47.46
Other	0	0	0	0.044	0
Cleaning of facilities					
With cleaning	26	3.85	0.2 - 21.6	0.044	0
Without cleaning	0	0	0	0	0
Total	26	3.85	0.2 - 21.6		

$_{95\ \%}$CI = $_{95\ \%}$ confidence interval for the seroprevalence; OR $_{95\ \%}$CI = $_{95\ \%}$ confidence interval for the odds ratio.

Seroprevalence by practice of facility cleaning

All owners reported that cleaning of premises was done daily and sometimes every third day. This practice was considered as a key variable given its importance at farm level, since MAP may remain viable in soil for weeks or even months (Díaz *et al*., 2005). Because most of the animals are in extensive systems, absence of seroconversion of other individuals in the affected flock may be due to the fact that the MAP seropositive buck had not passed the bacteria through the feces yet. Alternatively, other animals coexisting with the infected buck may be in the incubation period (Chávez *et al*., 2004; Diaz *et al*., 2005; Soberón, 2011).

Seroprevalence by productive stage

The only seropositive animal found in this study was a buck, thus, seroprevalence for this productive stage was 3.85 % ($_{95\ \%}$CI 0.2 - 21.6; Table 3). Although the survey showed that also females have been acquired as replacement does from other states, none of them was apparently infected. This finding differs from the results obtained by Kruze *et al*. (2007), that all seropositive animals were females in production, excluding the other productive stages. Since bucks usually remain for long periods of time in the flock, the relevance of having one MAP-seropositive buck is greater than that of having one seropositive animal in any other productive stage (Acha and Szyfres, 2003; Soberón, 2011). The fact that in this study only one buck tested positive to MAP and no other animal in other reproductive stage did, might be due to the low sensitivity of ELISA, that failed to identify all the positive cases (Díaz *et al*., 2005).

Seroprevalence by breed

The only seropositive animal was a Saanen buck, so seroprevalence for this breed was 2 % ($_{95\ \%}$CI: 0.11 - 12.24) (Table 3). According to Underwood *et al.* (2003), there are no previous studies demonstrating MAP affinity for any particular breed, because most of the work done has been conducted in dairy breeds such as French Alpine, Toggenburg and Saanen. This preference for dairy breeds is associated to the importance given to paratuberculosis as a cause of

economic losses resulting from decreased milk production; however, Underwood *et al.* (2003) found that most of the reactors were animals bred for meat production.

Seroprevalence by origin

The only seropositive buck found in this study was purchased outside the state of Veracruz and, in accordance to data collected in the epidemiological survey, it was brought from the state of Guanajuato. This represents a prevalence of 1.82 % for the out-of-state category (Table 3). Although seroprevalence is not high, this data supports the observations of Favila-Humara *et al.* (2007) and Soberón (2011), that the main source of infection of paratuberculosis is the entry of animals purchased in other states or countries to local flocks, particularly those coming from the USA. Moreover, Chávez *et al.* (2004) state that the absence of an official paratuberculosis-free requirement for animals imported into Mexico poses a threat to domestic flocks.

Seroprevalence by body condition

None of the sampled animals with low body condition was seropositive, so the seroprevalence for this category was 0 % ($_{95\ \%}$CI: 0 - 32.14; Table 3). Wasting is a common sign in diseased animals due to the progressive loss of body condition (Abalos, 2001); thus, it is possible to have a great number of animals suffering from this condition in a severely affected flock. Such situation was not observed in the present study, although data from the survey revealed 11 animals showing a decreased body condition, but the cause for it was not investigated.

Seroprevalence in animals affected by diarrhea

The only seropositive animal in this study did not show signs of diarrhea, so the seroprevalence for this category was 0.56 % ($_{95\ \%}$ CI: 0 - 53.71; Table 3). Since the disease follows a chronic course, it is not possible to observe conspicuous signs of disease until the goat is four or five years old. Berh and Collins (2010) state that the most frequently observed signs of paratuberculosis are edema, epiphora, lung sounds, wasting and diarrhea, although other authors (Chávez *et al.*, 2004; Favila-Humara *et al.*, 2007) argue that the only visible sign is profuse diarrhea in the terminal stages of the disease, leading to progressive emaciation. However, before this can be observed the infection must have been reactivated.

Tabla 3. Seroprevalence of goat paratuberculosis in flocks by productive stage, breed, provenance, diarrhea signs and body condition, in the central region of Veracruz, Mexico**.**

	No. of animals	Seroprevalence %	$_{95\ \%}$CI	Odds ratio	OR $_{95\ \%}$CI
Productive stage					
Replacement does	24	0	0 - 17.17	0	0
Pregnant does	22	0	0 - 18.5	0	0
Lactating does	35	0	0 - 12.32	0	0
Bucks	26	3.85	0.2 - 21.6	0.044	0 - 344.9
Weaned kids	2	0	0 - 80.21	0	0
Dry goats	4	0	0 - 60.42	0	0
Milking goats	69	0	0 - 6.57	0	0
Breed					
French Alpine	53	0	0 - 8.42	0	0
Toggenburg	25	0	0 - 16.6	0	0
Saanen	49	2	0.11 - 12.24	0.006	0 - 47.5
Crossbred	51	0	0 - 8.8	0	0
Provenance					
Born in farm	127	0	0 - 3.66	0	0
Out of state	55	1.82	0.1 - 11.00	25.84	0.04 - 1.7
Diarrhea signs					
Yes	5	0	0 - 53.71	0	0
No	177	0.56	0.03 - 3.58	0.31	0 -1.98
Body condition					
Low	11	0	0 - 32.14	0	0 - 0
Normal	171	0.59	0.03 - 3.73	0.64	0.02 - 21.7
Total	182	0.55	0.03 - 3.50		

$_{95\ \%}$CI = $_{95\ \%}$ confidence interval for the seroprevalence; OR $_{95\ \%}$CI = $_{95\ \%}$ confidence interval for the odds ratio.

CONCLUSION

Only one animal tested positive to MAP by indirect ELISA test. There were no risk or protective factors detected for goat paratuberculosis for any of the variables in the five municipalities studied. It is concluded that there is still a low distribution of caprine paratuberculosis among goat flocks in central Veracruz, Mexico.

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