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Research article

Seroprevalence of paratuberculosis in sheep of Nayarit, Mexico

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Abstract

Paratuberculosis is a disease caused by Mycobacterium avium subsp. paratuberculosis (MAP). In domestic ruminants, MAP infection is largely sub-clinical, but can result in chronic diarrhea leading to emaciation and death. A survey of MAP was carried out in a non-vaccinated sheep population from Nayarit, México to estimate seroprevalence and histopathological findings. The aim was also to estimate the intra-herd correlation (re) and design effect (D) of MAP seropositivity and to determine the association of the disease with some animal-level risk factors. Serum samples from 368 sheep older than 2 years in 38 herds were evaluated using an indirect ELISA assay. Eleven of the 38 herds had at least one seropositive animal and 19 animals with a total of 368 tested positive for MAP (5.6%). The histological alterations found were characterized by enteritis and granulomatous lymphadenitis indicating that the death of the animal was caused by MAP infection.

Keywords: *Mycobacterium avium subspecies paratuberculosis*; histopathology; ELISA

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Introduction

Paratuberculosis is a chronic granulomatous enteric infection, distributed world-wide, and affects wild and domestic ruminant species (Singh et al., 2009; Singh et al., 2010). Clinical paratuberculosis causes weight loss and diarrhea, does not respond to treatment, and leads to emaciation and eventually death or premature culling of females. The majority of infected animals remains latent or subclinical without ever developing evident signs of the disease or production decline (Sweeney, 2011). The main path of spreading of the disease is the fecal-oral route during the early months of life of the animals through the intake of colostrum, milk, grass or contaminated water (Bedolla et al., 2011). The epidemiology of Mycobacterium avium subspecies paratuberculosis MAP is complex, characterized by a long incubation period, the ability to infect and survive in multiple mammalian hosts, the ability to evade the host immune response, a latent period of a few months to several years, and a longer survival in the environment. These features, in addition to the lack of

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reliable diagnostic test have hampered eradication attempts, and control programs have had only moderate success. Even today, several epidemiological aspects of paratuberculosis remained unknown, and its eradication has not been achieved by any country or region worldwide (OIE, 2013).

There are only a few studies on paratuberculosis in Mexico, although it is reported to be present in all the country (Chávez et al., 2004). The studies in Mexico have been carried out on in temperate conditions, but no study has been reported under tropical conditions as such the state of Nayarit, where there is no report on the presence or prevalence of paratuberculosis in any wild or domestic species. The knowledge of the distribution, prevalence of paratuberculosis and risk factors associated are essential elements to establish prevention and control programs for this disease.

On the other hand, sample size and precision of cluster studies, where the herd and not the animal are the sampling units, are commonly influenced by the conditional probability of disease, therefore the estimation of the design effect are important to adjust for such bias (Solorio-Rivera et al., 2007; Solis-Calderon et al., 2007).

The objective of this study was to find the prevalence of paratuberculosis and histopathological changes in sheep herds in Nayarit, Mexico.

Materials and Methods

The state of Nayarit is located in North West Mexico, between coordinates 19°30' and 21°35' north and 90°43' and 105°46' west. Nayarit is bordered in the north with the states of Sinaloa and Durango, in the west with Durango, Zacatecas and Jalisco, in the south with Jalisco and the Pacific Ocean and in the west with the Pacific Ocean and Sinaloa (INEGI, 2000). Ninety-one point five percent of Nayarit is tropical subhumid with annual average temperature of 25°C, and minimum and maximum temperature is 12 and 35°C, in January and May, respectively. Most of the rain occurs during the months of May (annual average = 1100 mm).

Nayarit is classified into five regions: the Coast region that include the municipalities of Santiago and Tuxpan; the Centre-Southern region the municipalities of Compostela, San Pedro Lagunillas and San Blas; the South region that includes Ahuacatlán, Ixtlán del Rio, Santa María del Oro and Jala; the North region that includes Acaponeta, Tecuala and Huajicori, and the central region that includes the municipalities of Tepic and Xalisco. The main sheep production system is extensive, mainly based on year grazing on native pasture, and there are registered 475 sheep herds and 21,965 heads.

Study design

A stratified cross-sectional study with two stage sampling was carried out from June to December 2013, where herds were the initial sampling units. To determine the total number of animals to be sampled a prevalence of 10% was considered, using an infinite population size, a 95% confidence level, and a 3% precision (Schaeffer et al., 1987). In each herd only, adult animals (>2 years old) were sampled. However, considering that animal infection within herds were dependent an arbitrary design effect of 2 was used (Segura and Honhold, 2000). The sample size used was n= 368 adult sheep. The number of animals sampled within herd varied from 3 to 24 and the number of herds sampled was 38.

Laboratory analysis

Blood samples (10ml) were collected from the jugular vein of each animal, using disposable needles and Vacutainer tubes (Becton Dickinson^{R} Rutherford, NJ, USA) according to the Official Mexican Standard (NOM-062-ZOO-1999), and transported in ice box to the laboratory. The samples were centrifuged at 1500 g for 10 minutes to obtain the serum. Sera were stored in identified vials at -20°C until testing. Blood samples were tested for antibodies against MAP with an indirect enzyme-linked immunosorbent assay kit (IDEXX Laboratories, Inc., Westbrook, Maine USA), following manufacturer instructions. The sensitivity and specificity values of the ELISA assay were 53.6% and 98.9% respectively (Köhler et al., 2006; Gumber et al., 2006) and were used to calculate the true prevalence.

Histopatology

Eight suspected sheep were slaughtered and pieces of the large and small intestines, mesenteric lymphatic ganglia were taken, and fixed in 10% formalin tamponade. The collected tissues were analyzed using the usual histopathological techniques, included in paraffin, stained with Hematoxiline-Eosine. Slides prepared from tissue impressions were stained according to the Ziehl–Neelsen technique for the presence of acid-fast bacilli (Stabel, 2000; Simutis et al., 2005; Singh et al., 2013).

Potential risk factors

Data on potential risk factors were obtained using a questionnaire administered to the farmer or manager in each farm at the time of blood samples were obtained. Animal exposure variables were breed (Pelibuey, Khatadhin, Dorper, Blackbelly) sex (male or female), parity number (1, 2 3-5 and >5 lambing), body condition score (3-5), and whether or not the animal was born in the herd. Also, prevalence between five regions was compared.

Data analysis

Descriptive statistics were used to calculate the frequency of herds positive for antibodies against MAP. The results of all serum samples were also used to estimate the overall prevalence in the region. To determine the association of the risk factors here studied and the prevalence of MAP, fisher exact test was used because of small number of data on some cells. Post-hoc intra-herd correlation (re) for MAP infection was estimated from the components of variance of a one-way analysis of variance with herd as the only effect, and the design effect (D) was calculated as D = 1 + (k-1)re, where k is the average number of animals sampled per herd. All analysis was carried out using the SPSS package, version 9.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Herd and animal seroprevalence

Eleven out of the 38 herds had at least one seropositive animal and 19 sheep were ELISA seropositive. The apparent and true prevalences were 5.16% and 7.6%, respectively. None of the risk factors were associated with the seropositivity to MAP.

The seroprevalence per region is shown in Table 1. Of the animals sampled, 16 female out of 346 and 3 male out of 22 were found seropositive. The intra-herd correlation and the design effect for seroprevalence of MAP were both equal to 0.

Although region was not a significant risk factor for MAP, the lowest seroprevalence was found in the Costa and Centro regions and the highest in the Centro-Sur region.

Histopatology

The necropsied animals were 3 years old and showed progressive emaciation until death. A variable enlargement of the intestinal mucosa of the ileum and of the proximal portion of the small intestine was observed, particularly of the anterior part of the caecum. The mesenteric blood vessels were enlarged and congested. Also, a large amount of lymphocytes, mononuclear cells, infiltrating the submucosa and the epithelium (inflammatory granulomatosis cells), epithelial cells in large amounts, many of them in different stages of necrosis were observed (Fig. 1).

Under the Ziehl-Neelsen stain, resistant intra-cellular acid-alcohol bacilli were observed in red color at the middle of a blue background of the tissue cells (Fig. 2).

Discussion

Serological response, in this study, reflects natural exposure because vaccination of sheep against MAP was not practiced in Nayarit, Mexico. The low seroprevalence found in this study may be associated with the fact that in this study adult sheep were sampled. In cattle, it is known that young calves are more susceptible to MAP infection, especially in less than 30 days of age, with susceptibility declining with increasing age (Sweeney, 1996; Wells and Wagner, 2000; Mackintosh et al., 2010). However, resistance is

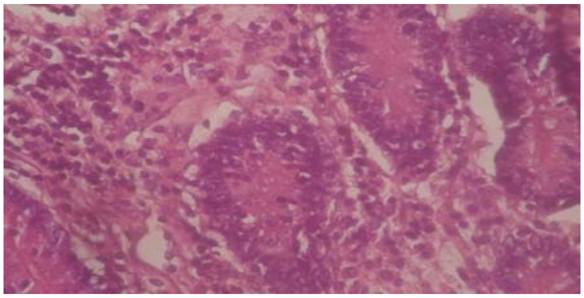

Fig. 1: Ileum, granuloma inflammation, lymphocytes, monocytes and epithelioid cells. Hematoxiline-Eosine staining.

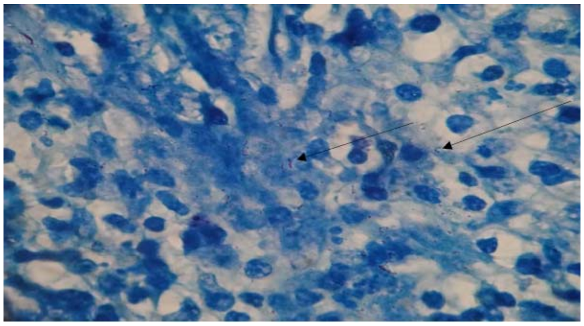

Fig. 2: Epithelial tissue of the intestine: Presence of acid-fast-bacilli (red color) in slides prepared from tissue impressions that stained with Ziehl–Neelsen technique.

Table 1: Seroprevalence by region for paratuberculosis in Nayarit, Mexico

Region	Number of herds	Animals sampled	Negative	Positive	Seroprevalence (%)
Costa	5	53	52	1	1.88*
Centro-Sur	7	66	59	7	10.6*
Sur	8	114	107	7	6.14
Norte	8	44	42	2	4.54
Centro	10	91	89	2	2.19*
Total	38	368	349	19	

*statistically significant values P<0.05

incomplete and animals could still be infected as adults if they stayed productive for a long period of years or were exposed to high challenge doses of MAP (Mackintosh et al., 2010).

The apparent (5.16%) and true (7.6%) seroprevalence here reported are within the range of 3.59-10% reported in small ruminants in other states of Mexico. Méndez et al. (2013) in Hidalgo, Mexico reported a prevalence of 3.59%, in goats. Moron-Cedillo et al. (2013) in San Luis Potosi, using immune-diffusion in gel agar (IDGA) reported a prevalence of 9.4% in sheep. Santillán et al. (2007), using IDGA estimated 4.33% (60/1385) in sheep. Jaimes et al. (2008) in Guanajuato and Aguascalientes, using PCR detected MAP prevalence of 9% in sheep; whereas, Estévez (2006) in Oaxaca and Veracruz reported 10% prevalence, in sheep and goats.

Also the seroprevalence here reported is within the values notified in Latin-America. In Colombia, Mancipe et al. (2009) reported 0.8% seropositivity in sheep using an ELISA assay. In Chile, Kruze et al. (2007) using fecal culture diagnosis confirmed by PCR reported 9.1% prevalence in goats. In Argentina, Jorge et al. (2000) reported prevalence of 7.2 to 19.6% in sheep. Higher seroprevalence values have been reported in The United States (40%) and in Australia 9-22% (CONASA, 2010). The differences among the prevalence reported may be due to differences in ecological conditions, climatic conditions, system of production, method of diagnosis, and age of the animal among others.

The r_e and D were both zero meaning independence and low transmission of MAP among sheep of a flock, and similar seroprevalence from herd to herd. This result disagreed with what is expected for a contagious disease such as paratuberculosis. However, this can be partially explained by the fact that susceptible animals are probably being challenged by reduced infectious doses of MAP, which did not allow for the detection of new positive animals.

Several studies have suggested that there is a dose–response relationship between the exposure to MAP and the severity and time to onset of clinical disease (Mackintosh et al., 2010; McGregor et al., 2012). The dose-response relationship between MAP challenge and the onset of clinical paratuberculosis has a strong implication in disease control programs. A successful control program will reduce infection prevalence and MAP burden, therefore it will become more difficult to detect infected animals because susceptible animals will be challenged by a reduced infectious dose, thus infected animals will seroconvert or start shedding MAP when older, delaying the detection of new positives (Taylor, 1953).

Mucosa and submucosa of the ileum, the lesions found were characterized by the presence of swollen infiltrates constituted by variables amounts lymphocytes, plasmatic cells, macrophages, epithelioid cells and the presence of resistant acid-alcohol bacilli clearly observed with the Ziehl-Neelsen staining agreed with the results by Stabel (2000), Simutis et al. (2005) and Singh et al. (2013).

Conclusion

This study shows that sheep in Nayarit are exposed to MAP, which is corroborated by the histological evaluation. The low prevalence of MAP may suggest low doses of MAP in susceptible animals. Under the conditions of this study the design effect was zero.

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