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Article · May 2022

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SEROLOGICAL IDENTIFICATION OF OVINE PARATUBERCULOSIS IN VERACRUZ, MEXICO

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AUTHORS’ CONTRIBUTIONS
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Juan Carlos Troiano, University of Buenos Aires, Argentina.
(2) Mohammadi, Zahedan University of Medical Sciences, Iran.

Reviewed:
(1) Leith Mohammadi, Zahedan University of Medical Sciences, Iran.
(2) Mr. David Mwenya Nakitare, Meru University of Science and Technology, Kenya.

Received: 02 March 2022
Accepted: 05 May 2022
Published: 09 May 2022

ABSTRACT

This study aimed to determine the presence of Mycobacterium avium subspecies paratuberculosis (MAP) in three sheep production regions in the state of Veracruz, México. The study regions were the Totonaca, Capital, and Tuxtlas. The study included 414 animals distributed in 55 farms. Ewes and males used as rams over three months old were randomly selected to collect serum for its subsequent analysis by indirect ELISA standard kits. Overall seroprevalence was 0.96% (IC95% 0.02-1.90), 23.07% (IC95% 0.017-45.98) by municipality, and 5.45% (IC95% 0.00-11.46) by flock. This could be the first report of exposition to MAP in sheep from the state of Veracruz. Since it is the third ovine producer in Mexico, the undetected threat of paratuberculosis (PTB) could induce economic losses and commercial disadvantage to producers. Meanwhile, the subclinical animals deemed as seropositive in this study, could be use as breed. Therefore, subclinical animals might maintain and disseminate the pathogen within herds. Furthermore, exposure to MAP was identified in the three regions studied (which have different climate conditions). These results confirm the environmental resistance of MAP and imply a health risk to ruminants and other susceptible species. Besides, in extensive livestock production, local wildlife could acquire MAP and transmit to other flocks or regions. None of the studied variables were

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considered as risk factor associated with MAP. Results suggest the need to develop further research to establish the distribution and impact of MAP in Veracruz. This work also prompts to enhance biosecurity measures amongst ovine producers.

**Keywords:** Epidemiology; enteritis; ELISA; ovine production.

1. **INTRODUCTION**

Paratuberculosis, also called Johne’s disease, is a chronic granulomatous enteritis caused by bacillus *Mycobacterium avium*, subspecies *paratuberculosis* (MAP) [1]. This species affects bovines [2], goats [3], ovines [1], camels [4], buffaloes, deers [5], and many other similar species. In sheep, the disease is transmitted by the fecal-oral route due to feces ingested from infected animals. However, it can also be passed from mother to offspring through the intrauterine route, milk, or colostrum [6]. Since there is no treatment, economic loss occurs in the industry. That is why several nations have implemented programs to identify and eliminate infected animals [7]. ELISA has been often used to establish control programs (2015): Bauman et al. [8] found high seroprevalence rates in small ruminants’ farms and described lack of knowledge among producers about the paratuberculosis status. In Mexico, high prevalence rates have been identified in family-run production systems [9]. Since surveillance is a key point to establish distribution of the pathogen [10], identification and report measures must be enhanced among countries toward implementing specific management practices to decrease its production and economic losses. Despite it is not currently considered a zoonosis, MAP has been linked to the Crohn’s disease in humans [11]. Although paratuberculosis (PTB) has worldwide impact on sheep, the epidemiological information in Latin American countries such as Mexico is scarce [12]. The state of Veracruz is the third ovine producer in Mexico, and in the last five years, ovine production has increased by about 6.66% [13]. This study aimed to determine the exposure to the agent in regions of Veracruz, Mexico, where sheep breeding is a growing practice.

2. **MATERIALS AND METHODS**

2.1 **Location**

The state of Veracruz, situated in eastern Mexico (Latitude 19°10.857’ N and Longitude 96°8.574’ W), has an area of 72,815 Km² and is divided in 212 municipalities. Nowadays, it is the third state with the highest sheep production, with 708,853 heads in 2019 [13].

The cross-sectional study was carried out in 13 municipalities distributed in 3 of 12 Rural Development Districts (Distritos de Desarrollo Rural, DDR) of the state office of the Department of Agriculture and Rural Development (Secretaría de Agricultura y Desarrollo Rural, SADER) in Veracruz (Fig. 1). The districts were selected based on its location (north, center and, south of the state) and their dedication to ovine production.

![Map](image)

**Fig. 1. Municipalities and Rural Development Districts considered in this study [14]**

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The Totonaca Region is in the north of Veracruz, in the DDR 02 Tuxpan, and the DDR 03 Martínez de la Torre. The altitude ranges between 20 and 180 masl, and the average temperature oscillates between 20.8 and 25.5°C. This region encompasses Gutiérrez Zamora, Papantla, Tlahualil, and Coatzintla, with 6.1% of the sheep population of the state. The Capital Region is in the DDR 04 Coatepec in the center of the state and includes Alto Tongo, Jalacingo, Perote, Ayahualulco, and Emiliano Zapata. This region contains 15.1% of the sheep inventory in Veracruz, its average temperature oscillates between 10 and 25.2°C, and the altitude ranges from 180 to 2,060 masl. The Tuxtla Region is in the south of Veracruz, in the DDR 09 San Andrés Tuxtla. It includes Santiago Tuxtla, San Andrés Tuxtla, Catemaco, and Angel R. Cabada, with 2.1% of the sheep population, the altitude varies from 10 to 340 masl, and the average temperature is between 23 and 25.3°C. Although the three regions studied represent only 23.3% of the ovine inventory, it is essential to emphasize that Veracruz state is vast, with 212 municipalities sprayed in 12 DDR. Therefore, the farm dispersion is also wide.

2.2 Study Design

The sample size was determined considering 50% expected prevalence of disease (N=695,507) with 95% confidence interval and 5% error margin. The minimum sample size obtained was 385 animals, and, according to the inclusion criteria, were sampled 414 animals. A pair of questionnaires were designed and applied to obtain information per animal (such as age, reproductive stage, presence of clinical signs) and per flock (size, productive system, water source). The animals considered for investigation were females over 3-month-old, rams, and their prospects. Furthermore, the characteristics of the farms were considered for classification in extensive, intensive, and semi-intensive. In the extensive livestock production, animals are grazing on local vegetation, kept together in a single flock and health, reproductive, and genetic control are lacking or deficient. Meanwhile, intensive programs are characterized because animals are fed on integral diets and good quality forages to ensure maximum performance and the highest reproduction efficiency; good health, reproductive, and genetic controls are kept. The semi-intensive is a combination of both: animals are kept between the farms and grazing at will in the pastures nearby.

2.3 Sampling

Sampling was carried out from August 2018 to May 2019. Ewes older than three months old, ram prospects, and rams were included. Blood samples were obtained by jugular vein puncture with vacuum tubes without anticoagulant and transported to the laboratory for their processing. Samples were centrifuged at 1,000 g for 15 minutes to separate the serum that was stored at -20°C until their serological analysis.

2.4 Serological Analysis

Samples were tested by indirect ELISA standard kits, available for serum, plasma, and milk (IDEXX Institut Pourquier, POURQUIER® ELISA Paratuberculosis “Antibody Screening, and Antibody Verification®, France). This modality of tests has an absolute sensitivity between 40.8 and 66% and an absolute specificity between 99 and 99.8%. The samples were adsorbed by M. phlei, which were diluted to a concentration of 1:20 for controls and sera and incubated at 21°C for one hour. Lately, they were transferred to 96-well polystyrene microplates coated with the M. avium paratuberculosis antigen, at a rate of 100 µL of controls and sera, and incubated at 21°C for 45 minutes, and then washed three times with buffer solution. After 10 minutes, the reaction was stopped with 100 µL of stop solution. An ELx800 plate reader (BioTek®) was utilized to read the samples at an absorbance of 450 nm, and the results were interpreted by the XCheck® program.

2.5 Statistical Analysis

Overall prevalence, per municipality and by flock were calculated with the formula proposed by Thrusfield [15]. The proportion of ELISA test positive is calculated dividing the number reacting animals by the number of sampled individuals. The IC₉₉ were calculated by the online program WinEpi. Odds ratio (OR) was calculated to obtain the association between risk factors and the disease using the Vassarstats online program.

3. RESULTS

Out of 414 sampled animals, showed antibodies against MAP, indicating and overall seroprevalence of 0.96 % (IC₉₉: 0.02–1.90%). Out of all the sampled animals (n=414), 330 (79%) were females, and 84 (20%) were males (Table 1). X² test associated value was 2.20 (p= 0.1376).

Seropositive animals were identified in the three regions considered for this study (Table 2). Odds ratio analysis shows no statistical association between variables previously related to MAP and possible risk factors linked to PTB.
Table 1. Seroprevalence (%) of ovine paratuberculosis, per municipality and flock in the main production areas of Veracruz

<table>
<thead>
<tr>
<th>Seroprevalence</th>
<th>Sampled Animals</th>
<th>Positive Animals</th>
<th>Seroprevalence (%)</th>
<th>IC95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>414</td>
<td>4</td>
<td>0.96</td>
<td>(0.02–1.90)</td>
</tr>
<tr>
<td>Municipality</td>
<td>13</td>
<td>3</td>
<td>23.08</td>
<td>(0.17–45.98)</td>
</tr>
<tr>
<td>Flock</td>
<td>55</td>
<td>3</td>
<td>5.45</td>
<td>(0.00–11.46)</td>
</tr>
</tbody>
</table>

Table 2. Seroprevalence of ovine paratuberculosis and risk association analysis

<table>
<thead>
<tr>
<th>Categories</th>
<th>Sampled Animals</th>
<th>Positive Animals</th>
<th>Seroprevalence (%)</th>
<th>IC95%</th>
<th>OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Region</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totonaca</td>
<td>68</td>
<td>1</td>
<td>1.47</td>
<td>(0.00–4.33)</td>
<td>2.80</td>
<td>0.45</td>
</tr>
<tr>
<td>Tuxtlas</td>
<td>157</td>
<td>2</td>
<td>1.27</td>
<td>(0.00–3.03)</td>
<td>2.42</td>
<td>0.43</td>
</tr>
<tr>
<td>Capital</td>
<td>189</td>
<td>1</td>
<td>0.52</td>
<td>(0.00–1.56)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td><strong>Municipality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tihuatan</td>
<td>16</td>
<td>1</td>
<td>6.25</td>
<td>(0.00–18.11)</td>
<td>2.33</td>
<td>0.52</td>
</tr>
<tr>
<td>San Andres</td>
<td>35</td>
<td>2</td>
<td>5.71</td>
<td>(0.00–13.40)</td>
<td>2.12</td>
<td>0.48</td>
</tr>
<tr>
<td>Tuxtlas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jalacingo</td>
<td>36</td>
<td>1</td>
<td>2.78</td>
<td>(0.00–8.15)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td><strong>System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensive</td>
<td>40</td>
<td>2</td>
<td>5.00</td>
<td>(0.00–11.75)</td>
<td>6.26</td>
<td></td>
</tr>
<tr>
<td>Extensive</td>
<td>240</td>
<td>2</td>
<td>0.83</td>
<td>(0.00–1.98)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Semi-intensive</td>
<td>134</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>84</td>
<td>2</td>
<td>2.38</td>
<td>(0.00–5.64)</td>
<td>4.0</td>
<td>0.18</td>
</tr>
<tr>
<td>Females</td>
<td>330</td>
<td>2</td>
<td>0.60</td>
<td>(0.00–1.44)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Reproduction state</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating ewe</td>
<td>27</td>
<td>1</td>
<td>3.70</td>
<td>(0.00–10.83)</td>
<td>5.26</td>
<td>0.30</td>
</tr>
<tr>
<td>Rams</td>
<td>84</td>
<td>2</td>
<td>2.38</td>
<td>(0.00–5.64)</td>
<td>3.34</td>
<td>0.32</td>
</tr>
<tr>
<td>Pregnant</td>
<td>138</td>
<td>1</td>
<td>0.72</td>
<td>(0.00–2.14)</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

4. DISCUSSION AND CONCLUSION

According to Espeschit et al. [12], that describes the low PTB epidemiologic studies in Latin American countries, this seems to be the first known report about exposure to MAP in sheep in Veracruz state, which is the third-highest sheep state producer in Mexico [13]. The general seroprevalence found in this study was lower than in other Mexican states, such as Nayarit with 7.6% in and Sonora with 7.48% [16]. However, Morales- Pablos et al. [16] used immunodiffusion in agar gel, which has a different sensibility than the ELISA test. Based on the ability of ELISA test to detect affected animals, the seropositive sheep that were identified suggest they only represent just the tip of the iceberg. It implies an unnoticed health problem within Veracruz flocks that impacts production and represents economic losses and commercial disadvantages. As Whittington et al. [10] mentioned, ‘economic losses due to paratuberculosis is a key driver to control MAP’. Nonetheless, Veracruz lacks PTB surveillance programs. Since ELISA test is a fundamental strategy for disease identification [17], this work could be a basis to establish PTB presence and, if necessary, to develop a control and eradication program. On the other hand, when a serologic diagnosis of the disease is made, they might have had already infected other susceptible individuals, including different animal species, through contaminated water or grass [18]. Therefore, if local susceptible wildlife has been infected, it could transport the agent through farms and affect sheep, goats, cows, and buffaloes.

In this study, seropositive animals were identified in three municipalities considered for investigation. These results confirmed that MAP has a high resistance to environmental conditions [19] because the farms are in regions with different climatic and geographical conditions, ranging from a minimum temperature of 10°C to a maximum of 25.4°C. Thus, the variations are like those observed by Kumthekar et al. [20], who reported seroprevalence of 0, 1.3, and 3.2% in distinct sheep production units in Grenada. These differences might imply that some geographical
conditions have an impact on the distribution of the pathogen.

The seroprevalence in intensive and extensive production systems was 5.00 and 0.83%, respectively (Table 2). Similarly, to this study, Giannitti et al. [21] reported a seroprevalence of 1.23% in extensive grazing flocks in Colonia, Uruguay. However, analyzed animals showed clinical signs compatible with Johne’s disease (emaciation, submandibular edema, and intermittent diarrhea); meanwhile, the seropositive sheep in our study were asymptomatic. Since antibody ELISA identifies animals more often as the infection progressed [18] and, because the progression of the infection in sheep is mostly late, the development of clinical manifestation suggests a higher antibody response, in comparison to the animals that did not show them. Because extensive livestock production is common in Veracruz, and animals in these conditions are freely grazing, they are exposed to the main route of infection of PTB, it is essential to recognize and replace infected sheep to prevent the spread of MAP. Meanwhile, extensive management implies deficiency or lack of genetic control within the flocks. Also, producers can purchase sheep from other farms, increasing the risk of transmission thoroughly infected subclinical animals. The external provenance of sheep has been reported as a risk factor associated with MAP [16]. Some farms have deficient sanitary measures (absence of quarantine when acquiring external animals, poor hygiene, inadequate corpse disposal, lack of control at the entrance and exit of the facilities). These insufficient biosecurity strategies may maintain MAP among sheep from the same farm. Furthermore, this absence of sanitary measures could mean insufficient veterinary advisory for the producers. If so, it is an indicator of the need to reinforce programs focused on raising awareness about the importance of biosecurity. No animal was identified as seropositive inside the semi-intensive farms.

On the other hand, two ewes seropositive to MAP were identified, one was pregnant, and the other was a lactating ewe. Although sporadic, these animals pose an infection risk because the agent transmission is widespread through the intrauterine route, colostrum, suckling, and contaminated udders [22]. Moreover, it has been identified the presence of MAP in milk of ewes coming from flocks without seropositive animals [2]. This situation constituted a potential threat because whether infected lambs are not detected they might disseminate the agent. In this case, when an infected animal is sold as ram prospects or as reproducers, it could transmit MAP through farms and in other municipalities or regions. Two of the seropositive animals identified were used as rams and stay for long periods in the farms. Therefore, if males were not diagnosed as MAP carriers, they could keep the pathogen through several years amongst flocks.

Although MAP is not considered as zoonosis, the subject is still inconclusive. MAP has been related to idiopathic intestinal inflammatory disease in humans [23]. The potential hazard of MAP in public health makes it essential to determine if it is true. If so, biosecurity measures must be improved to avoid human infection.

In conclusion, ovine production is increasing in Veracruz; however, our results expose the presence of MAP within sheep flocks, highlighting the impact of this disease and its distribution among farms. Therefore, it is crucial to identify the quality of biosecurity measures in ovine production. If these are deficient, it could lead not only PTB outbreaks but to other emerging diseases. Moreover, this information might suggest the need to enhance epidemiological surveillance programs in Veracruz and other Mexican states.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

AVAILABILITY OF DATA AND MATERIAL

Data are available with the corresponding author upon reasonable request.

CONSENT TO PARTICIPATE

As per international standard or university standard, Participants’ written consent has been collected and preserved by the author(s).

ACKNOWLEDGEMENTS

This study was financed by Produces Veracruz Foundation (FUNPROVER) and developed in the Laboratory of Microbiology, Faculty of Veterinary Medicine, Universidad Veracruzana as a first stage of the doctoral project carried out by the first author.
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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