Seroprevalence of *Mycobacterium avium* subspecie paratuberculosis in suspect cattle slaughtered at slaughterhouse from Sonora, Mexico

Seroprevalencia de *Mycobacterium avium* subspecie *paratuberculosis* en bovinos sospechosos sacrificados en rastro de Sonora, México

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**ABSTRACT**

Paratuberculosis is an infectious-contagious disease caused by the bacterium *Mycobacterium avium* subspecie *paratuberculosis* (MAP), which affects the digestive tract of ruminants. MAP is distributed worldwide; however, there are no publications on the seroprevalence of this disease in cattle in Sonora State. Therefore, the objective of this study was to detect MAP seropositivity in cattle in Sonora, as well as to know its apparent prevalence in a population of adult cattle with emaciation and cachexia. Three hundred and eighty-five serum samples were analyzed from 370 female and 15 male cattle, older than 3 years, born in the state with signs of emaciation and cachexia. Data were collected on age, breed, zootechnical purpose and municipality of origin. The zootechnical purpose of the animals was mostly dual-purpose cattle with 84.93% (370/385). The samples were processed using the commercial ELISA test. The results show an apparent seroprevalence of 2.08% (8/385) and municipalities that presented seropositive animals were Hermosillo with 3 samples, as well as Guaymas, Huachinera, Ures, Villa Pesqueira and Fronteras, with one sample each. The present study demonstrates for the first time seropositivity to *Mycobacterium avium* subspecies *paratuberculosis* in cattle in Sonora. In addition to a low apparent seroprevalence in the cattle population sampled. The presence of MAP in Sonora, which could be considered a neglected disease, highlights the need to know its possible role in public health in Mexico.

**Keywords**: Seroprevalence, *Mycobacterium avium* subspecie *paratuberculosis*, Sonora.

**RESUMEN**

La paratuberculosis es una enfermedad infecto-contagiosa causada por la bacteria *Mycobacterium avium* subspecie *paratuberculosis* (MAP), y afecta al aparato digestivo de los rumiantes. MAP es de distribución mundial, sin embargo, no hay publicaciones de la seroprevalencia en bovinos de esta enfermedad en el estado de Sonora. Por lo anterior, el objetivo de este trabajo fue detectar la seropositividad a MAP en ganado bovino de Sonora, así como conocer su prevalencia aparente en una población de bovinos adultos con emacación y caquexia. Se analizaron 385 muestras de suero de bovinos, 370 de hembras y 15 de machos, mayores de 3 años, nacidos en la entidad con signos de emacación y caquexia. Se recopilaron datos sobre edad, raza, fin zootécnico y municipio de origen. La finalidad zootécnica de los animales fue en su mayoría bovinos de doble propósito con 84.93% (370/385). Las muestras procesaron mediante la prueba comercial ELISA. Los resultados muestran una seroprevalencia aparente de 2.08% (8/385) y los municipios que presentaron animales seropositivos fueron Hermosillo con 3 muestras, así como, Guaymas,
Huachinera, Ures, Villa Pesqueira y Fronteras, con una muestra cada uno. El presente estudio demuestra por primera vez la seropositividad a Mycobacterium avium subespecie paratuberculosis en ganado bovino de Sonora. Además de una baja seroprevalencia aparente en la población de bovinos muestreada. La presencia de MAP en Sonora, la cual podría ser considerada como una enfermedad olvidada, pone en evidencia la necesidad de conocer su posible rol en la salud pública de México.

**Palabras clave:** Seroprevalencia, Mycobacterium avium subespecie paratuberculosis, Sonora.

**INTRODUCTION**

Paratuberculosis (PTBC) or Johne's disease is a chronic and contagious disease caused by the bacterium Mycobacterium avium, subspecies paratuberculosis (MAP). In the agreement through which the exotic and endemic diseases and pests of compulsory notification of terrestrial and aquatic animals in the United Mexican States are made known in the Official Gazette of the Federation that was published in November 2018. PTBC is cataloged within group 3 of diseases and pests, which are constituted by those found in national territory, considered endemic, which represent a lower risk from the epidemiological, economic, public health point of view and for national and international trade; since they can be controllable and they are of obligatory monthly notification (DOF, 2018). MAP infection affects cattle, sheep and goats, mainly dairy cattle (Retamal *et al*., 2011; OIE, 2020). It causes granulomatous lesions in the digestive tract and it is characterized by causing proliferative granulomatous enteritis and lymphadenitis with mononuclear infiltration, which causes malabsorption syndrome in infected animals, affecting the productive performance of the animals (Rathanaiah *et al*., 2017). One of the main mechanisms of infection occurs in calves, when they were infected during lactation through oral-fecal infestation; although it is not ruled out that they could become infected in the stabling stage.

The incubation period of MAP ranges from 4 months to 15 years, and clinical signs may appear between two and five years of age (Eisenber *et al*., 2012). Due to the pathophysiological similarities between PTBC in ruminants and Crohn's disease in humans, it has been suggested that it could be a zoonotic disease (Naser *et al*., 2004); however, a direct link between MAP infection and Crohn's disease in humans has so far not been demonstrated (Chaubey *et al*., 2017). PCR of the IS900 gene has been performed and the actual prevalence of MAP in patients affected by Crohn's disease and non-inflammatory bowel disease was 47 and 13 % respectively; only 7 % of patients with Crohn's disease were moderately positive to Zielh Nelseen staining. Histopathology findings showed granulomatous enteritis (83 and 90 %), lymphoplasmacytic enteritis (17 and 14 %), edema and lymphangiectasia (67 and 96 %) and vasculitis (20 and 73 %), for Crohn's disease and MAP, respectively (Zarei *et al*., 2019).
Diagnosis of PTB can be by ELISA, PCR, bacterial isolation, complement fixation, agar gel immunodiffusion; as well as Ziehl Neelsen staining of histopathology and stool samples; it can also be performed by detection of cell-mediated immunity (anus caudal skin test); however it cross-reacts with *Mycobacterium bovis* (Whittington *et al.*, 2019). PTBC is distributed worldwide, in a survey of 48 countries it was found that PTBC is very common, in about half of these countries; more than 20% of herds were infected with MAP (Whittington *et al.*, 2019). Only Sweden, Norway and some states in Australia have been shown to be free of the disease, because valid reporting and control measures for MAP are carried out in these places, and because the goal is eradication of the disease (Collins *et al.*, 2010; Eslami *et al.*, 2019; Whittington *et al.*, 2019). PTBC presents a prevalence in cattle worldwide between 7 to 60 %, being lower in beef cattle than in dairy cattle; in addition, it has been estimated that in sheep it is 16 % in Latin America and the Caribbean (Fernández *et al.*, 2014).

In Mexico, the information available on MAP seroprevalence in cattle is scarce; the studies carried out have been mainly in sheep and goats. In sheep in San Luis Potosí a seropositivity of 9.48% was detected (Morón *et al.*, 2013); while in Jalisco, Guanajuato and the state of Mexico it was 44.6% (Jaimes *et al.*, 2008), in Nayarit 5.16% (Mejía *et al.*, 2017), in Baja California 7.8% (Correa *et al.*, 2013). In Sonora, an overall seroprevalence for sheep of 7.48% was reported and when analyzed by region, the highest seropositivity was observed in the south of the state with 11.10% (Morales *et al.*, 2020). Other studies in Mexico have reported the presence of MAP in goats, in Veracruz a seropositivity of 0.6% was detected (Martínez *et al.*, 2012), in the central part of Mexico of 8.29-9.67% (Favila *et al.*, 2010) and in Puebla a seroprevalence of 48.1% was found (Gallaga *et al.*, 2017). In the study conducted by Morales *et al.* (2020), risk factors for the presentation of seropositivity to MAP in sheep in Sonora were analyzed and it was found that the risk was higher when the animals were born outside the farm and in farms with more than 300 animals. Although information on MAP in cattle is limited, a seroprevalence of 8.29-9.67% has been observed in central Mexico (Favila *et al.*, 2010).

In a study conducted by Milián *et al.* (2015), where they analyzed the seroprevalence of MAP in cattle from different Mexican states, a seroprevalence of 5% was detected for the country; and states that showed the highest seroprevalence were Jalisco and Guanajuato, with 11% and 10%, respectively. This same study also included Chihuahua and Sinaloa states, which border Sonora, and detected a MAP seroprevalence of 2% and 6%, respectively. Unfortunately, there is no information available on the current situation of MAP in cattle in Sonora, but the presence of the disease in sheep from this state, as well as in sheep and cattle from neighboring states, suggests that the disease could be circulating in cattle in Sonora stat.
The objective of this work was to detect the presence of seropositivity to *Mycobacterium avium* subspecies *paratuberculosis* in cattle from different Sonora municipalities, as well as to know the apparent prevalence in a population of clinically suspect cattle (with emaciation and cachexia) and older than 3 years of age.

**MATERIAL AND METHODS**

Study design, sample size and collection.

A transversal observational epidemiological study was conducted between May 2018 and May 2019. The sample size (n=385), was determined with the methodology described by Segura and Hondhold (2015); for this, the total population of 1,728,001 cattle in Sonora state was considered: 1,709,366 beef and 18,635 dairy cattle (SIAP, 2018), with a confidence level of 95 %, an estimated error of 5 % and an expected prevalence of 5 %. Non-probabilistic convenience sampling was performed. The animals were selected from a TIF slaughterhouse in Hermosillo city, which receives cattle from all over the state of Sonora. Inclusion criteria were that the animals had a SINIIGA ear tag and a shoe brand that identified them as coming from Sonora, that they were older than 3 years, and that they showed signs of emaciation and cachexia. The exclusion criterion was that samples would not be collected from animals under 3 years of age and with body condition 3-5. In addition, data were collected on the zootechnical purpose of animals, age, sex and municipality to which they belonged. To classify the samples according to the geographic location from which the animals came, it was used the State Information System for Land Management of Sonora State (SEIOT), which groups different municipalities into territorial units, shown in Figure 1 (SEIOT, 2020).
Sample collection and processing
Blood samples were collected from the jugular vein of each animal in 10 mL tubes without anticoagulant (Vaccutainer®); the procedure was performed according to NOM-062-ZOO-1999. The detection of anti-MAP antibodies was performed using the indirect ELISA technique (Jark et al., 1997), with a commercial kit (IDEXX Laboratories, Inc., USA), and were performed according to the manufacturer’s instructions. The measurement was performed in an ELISA reader (Stat Fax® 4700), at an absorbance of 450 nm. Samples with an S/P > 55%, doubtful from 45 to 55 % and negative < 45 % were considered positive.

Data analysis. To determine the apparent seroprevalence of MAP in the cattle population tested (emaciation and wasting and emaciation adults), the animals positive for MAP by ELISA were considered and divided by the total number of the population sampled.

RESULTS AND DISCUSSION

A total of 96.10 % (370/385) females and 3.90 % (15/385) males were sampled. It was found that 3.90 % (15/385) of the animals sampled were beef cattle, 84.93 % (327/385) dual purpose (meat and milk production) and 11.17 % (43/385); the zootechnical purpose was milk production. The average age of MAP seropositive animals was 7.5 ± 2 years, according to the literature and MAP infection usually occurs in the first months of life of animals, in the lactation stage by milk contaminated with feces, but clinical signs are observed after a long incubation period and generally appear between two and five years (Craven et al., 2000). The animals sampled in this study were mainly cull cattle; they also presented the characteristic signs of PTBC, which are emaciation and cachexia. Although the presumptive diagnosis of the disease can be made based on clinical signs and necropsy findings, there are other diseases that have the same clinical manifestations; therefore, laboratory confirmation and serological tests are required (Bustamante et al., 2011).

Serological samples were obtained from cattle from 55 of the 72 municipalities in Sonora state (Table 1), which corresponds to 76.39 % of the state territory; these municipalities are located in the north-central part of the state (Fig. 1).

The municipality with the highest number of samples was Hermosillo with 45.45 % (175/385). In the serological analysis for the detection of anti-MAP antibodies, it was observed that the municipalities where doubtful samples were detected were Hermosillo (n = 3), Guaymas (n = 1), Huachinera (n = 1), Ures (n = 1), Villa Pesqueira (n = 1) and Fronteras (n = 1) (Fig. 2).
Table 1. Municipalities of Sonora State evaluated in the study

<table>
<thead>
<tr>
<th>Basic Territorial Unit</th>
<th>No. of animals</th>
<th>Municipalities included</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hermosillo</td>
<td>175</td>
<td>Hermosillo, San Miguel de Horcasitas, La Colorada, San Javier, Carbo</td>
</tr>
<tr>
<td>Guaymas</td>
<td>13</td>
<td>Guaymas, Empalme</td>
</tr>
<tr>
<td>Cajeme</td>
<td>12</td>
<td>Cajeme, Suaqui Grande, Rosario, Quiriego, San Ignacio Río Muerto, Bácum</td>
</tr>
<tr>
<td>San Luis Río Colorado</td>
<td>2</td>
<td>San Luis Río Colorado, Puerto Peñasco, Gral. Plutarco Elías Calles</td>
</tr>
<tr>
<td>Caborca</td>
<td>16</td>
<td>Caborca, Pitiquito, Saric, Íltar, Tubutama, Átil, Oquitoa</td>
</tr>
<tr>
<td>Nogales</td>
<td>23</td>
<td>Nogales, Santa Cruz, Ímuris, Magdalena, Santa Ana, Benjamín Hill, Trincheras, Cucúrpe</td>
</tr>
<tr>
<td>Agua Prieta</td>
<td>11</td>
<td>Agua Prieta, Fronteras, Nacozari de García, Bacoachi, Cananea, Naco</td>
</tr>
<tr>
<td>Moctezuma</td>
<td>49</td>
<td>Moctezuma, Bacerac, Huachinera, Nacori Chico, Bacadehuachi, Huasabas, Granados, Divisaderos, Cumpas, Bavispe, Villa Hidalgo, Tepache</td>
</tr>
<tr>
<td>Banámichi</td>
<td>8</td>
<td>Arizpe, Banamichí, Huepac, Aconchi, Baviacora, San Felipe de Jesús</td>
</tr>
<tr>
<td>Sahuaripa</td>
<td>24</td>
<td>Sahuaripa, Arivechi, Yecora, Bacanora, Soyopa, Onavas</td>
</tr>
<tr>
<td>Ures</td>
<td>49</td>
<td>Ures, Opodepe, Rayón, Villa Pesqueira, Mazatán, San Pedro de la Cueva</td>
</tr>
<tr>
<td>Navojoa</td>
<td>3</td>
<td>Álamos, Etchojoa, Navojoa, Benito Juárez, Huatabampo</td>
</tr>
</tbody>
</table>
There are several studies of seroprevalence of the disease, where samples with not reliable results are considered positive (Velez et al., 2016; Jaramillo et al., 2017). The estimated apparent seroprevalence for the state was 2.08 % (8/385); these results coincide with that detected in Chihuahua by Milián et al. (2015). It is suggestive to note that, although Sonora also borders Sinaloa and Baja California, where higher seroprevalences were detected (Milián et al., 2015), the introduction of cattle into the state is prohibited due to its high sanitary status; this is because Sonora is in the eradication stage of Mycobacterium bovis (Bovine Tuberculosis). Among the official tests for the diagnosis of bovine tuberculosis in Mexico, the tuberculinization test is used, which are considered the ano-caudal fold test, comparative cervical and simple cervical. This test is highly sensitive and has low specificity, and the anus-rectal skin test is ideal to identify populations reacting to mycobacteria; therefore, to discriminate to whom this reaction could correspond, the double cervical comparative test is used (NOM-031-ZOO-1995).

There are immunogenic similarities of MAP with Mycobacterium bovis that a sweep was made with the tuberculin test through the joint U.S.-Mexico Strategic Plan for collaboration in bovine tuberculosis 2013-2018, and that the state of Sonora has advanced as accredited modified advanced for the USDA (United States Department of Agriculture). The state strategy is based on the elimination of reactors and depopulation of herds positive to histopathology tests and bacterial isolation (SENASICA, 2021). This has
allowed great progress in the bovine tuberculosis campaign and indirectly minimized the presence of MAP in cattle in the state. There is evidence of seropositivity for MAP in sheep in the state (Morales et al., 2020); this increases the possibility that there is the presence of circulating MAP antibodies in the state and that at some point cattle have contact with the disease.

CONCLUSIONS

The results obtained in the present study demonstrate for the first time seropositivity to Mycobacterium avium subspecies paratuberculosis in cattle in Sonora. In addition, a low apparent seroprevalence was observed in the cattle population sampled. However, in order to have more information on the epidemiological behavior of this etiological agent in the cattle population of Sonora, it is necessary that in the future more analyses be carried out in productively active animals within the production units. On the other hand, the presence of MAP in Sonora, which is a neglected disease, highlights the need to know its possible role in public health in Mexico.

CITED LITERATURE


