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Brief communication

Detection of *anti-Mycobacterium avium* subsp. *paratuberculosis* antibodies in wild deer in Colombia

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ABSTRACT

Objective. To determine the presence of anti-*Mycobacterium avium* subsp. *paratuberculosis* (MAP) antibodies in white-tailed deer (*Odocoileus virginianus*) and red brocket deer (*Mazama rufina*), captured in the Orinoquía and Caribbean regions in Colombia. **Materials and methods**. A total of 44 blood serum samples from deer of species *M. rufina* and *O. virginianus* were collected under field conditions between 2014 and 2016. An ELISA commercial kit was used to detect anti-MAP antibodies. An animal was considered ELISA-positive at a sample-to-positive ratio (S/P%) of ≥ 0.4, as recommended by the manufacturer. **Results.** The 50% (22/44) of the animals were positive, corresponding to 10 females and 12 males. An 81.8% (18/22) and 77.3% (17/22) of these seropositive animals were captured in the Orinoquía region and were reported as adults, respectively. **Conclusions.** It is not known how or when MAP was introduced in the Colombian deer population in the study regions. The most plausible hypothesis to explain the presence of antibodies against MAP in these wild populations is transmission by contact with infected bovine cattle since, in both regions, these species share pastures. This is the first study to explore MAP infection in wild animals in Colombia. These findings support the need for further studies using different direct diagnostic techniques and research approaches that allow the definition of links in the infection dynamics between wild and domestic mammals in Colombia.

Keywords: Cervids; ELISA; Johne's disease; micobacteria; wild ruminants (*Fuentes: DeCS, CAB*).

RESUMEN

Objetivo. Determinar la presencia de anticuerpos anti-*Mycobacterium avium* subsp. *paratuberculosis* (MAP) en venados de cola blanca (*Odocoileus virginianus*) y venados de páramo (*Mazama rufina*), capturados en las regiones de la Orinoquía y el Caribe en Colombia. **Materiales y métodos.** Un total de 44 muestras de suero sanguíneo de venados de las especies *M. rufina* y *O. virginianus* fueron colectadas en condiciones de campo entre 2014 y 2016. Se utilizó un kit comercial de ELISA para la

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detección de anticuerpos anti-MAP. Un animal se consideró positivo a ELISA cuando la relación muestra-positivo (S/P%) fue ≥ 0.4, según lo recomendado por el fabricante. **Resultados.** El 50% (22/44) de los animales muestreados resultaron positivos, lo que corresponde a 10 hembras y 12 machos. Un 81.8% (18/22) y un 77.3% (17/22) de estos animales seropositivos fueron capturados en la región de la Orinoquía y fueron reportados como adultos, respectivamente. **Conclusiones.** No se sabe cómo o cuándo se introdujo MAP en la población de ciervos colombianos en las regiones de estudio. La hipótesis más plausible para explicar la presencia de anticuerpos anti-MAP en estas poblaciones silvestres es la transmisión por contacto con el ganado bovino infectado, ya que en ambas regiones estas especies comparten pasturas. Este es el primer estudio en explorar la infección por MAP en animales silvestres en Colombia. Estos hallazgos respaldan la necesidad de realizar más estudios utilizando técnicas de diagnóstico directo, y aproximaciones investigativas que permitan la definición de vínculos en la dinámica de la infección entre mamíferos silvestres y domésticos en Colombia.

Palabras clave: Cérvidos; ELISA; enfermedad de Johne; micobacteria; rumiantes silvestres (*Fuentes: DeCS, CAB*).

INTRODUCTION

Mycobacterium avium subsp. *paratuberculosis* (MAP) is part of the *Mycobacterium avium* complex (MAC) and it is the etiological agent of a chronic gastroenteritis in ruminants known as paratuberculosis (PTB) or Johne's disease (JD). The PTB causes inflammatory lesions on the walls of the jejunum and ileum, affecting the absorption of nutrients and proteins, leading to loss of muscle mass and lower productivity. Affected bovines show diarrhea, submandibular edema, weight loss (despite normal appetite), lethargy, and death. In general, the clinical signs of PTB in cervids are similar to those observed in bovine cattle, namely chronic diarrhea, weight loss, and decreased body condition (1,2). The PTB leads to economic losses to livestock systems because of premature culling, replacements costs, reduced milk yield and weight gain, fertility disorders, and increased susceptibility to other diseases (3).

The potential association of MAP with Crohn's disease (CD) and other human diseases, reflects the zoonotic potential of the agent, recognized as associated to MAP (4).

Furthermore, PTB affects domestic and wildlife species worldwide (5). MAP has been confirmed in 178 captive and free-ranging wildlife species worldwide (6). There are reports to this respect in Latin America. One study reported the presence of MAP in feces of wild guanacos (*Lama guanicoe*) using microbiological and molecular techniques (7). In 2013, the first case of *Mycobacterium intracellulare* infection, a member of the MAC complex, was reported in a capybara (*Hydrochoerus hydrochaeris*) with granulomatous lesions typical of mycobacteriosis in Argentina, suggesting the susceptibility to infection by other bacteria of the MAC complex such as MAP (8). In Perú, samples from captive and free-ranging vicuñas (*Vicugna vicugna mensalis*) and others South American camelids (*Vicugna pacos* and *Lama glama*) were collected, reporting negative serological results to MAP (9). In a more recent study, the presence of MAP was reported in feces from Chilean alpacas (*V. pacos*) using culture, polymerase chain reaction (PCR), and genotyping techniques (10). In Colombia, the PTB was first reported in bovine cattle in 1924. Since then, the disease has been explored in cattle and sheep, but no epidemiological information on MAP in wild animals is available so far (11).

The red brocket deer (*Mazama rufina*) is native of the Colombian Andes, Ecuador, and of Northern Peru, found in forest and paramo environments. The white-tailed deer (*Odocoileus virginianus*) has a wider geographic distribution (from Canada to Peru) and eight subspecies are recognized in South America. The species *M. rufina* and *O. virginianus* provide an alternative source of protein in developing countries and are a highly prized hunting species, despite being a prohibited practice in Colombia (12).

The aim of the present study was to determine the presence of anti-MAP antibodies in white-tailed deer (*O. virginianus*) and red brocket deer (*M. rufina*), captured under field conditions in the Orinoquía and Caribbean region in Colombia.

MATERIALS AND METHODS

Ethics. The ethics committee for animal experimentation (CEEA) of the Universidad de Antioquia, Colombia (Act # 111, June 2017) approved this study. The national authority of environmental licenses (ANLA), Colombia (Auto # 00852, March 2017) approved the sample collection.

Animals and samples. Blood was collected in a convenience sampling from 44 individuals of free-ranging wild red brocket (*M. rufina*) and white-tailed deer (*O. virginianus*), captured and sampled under field conditions in the Orinoquía and Caribbean regions, between 2014 and 2016. A total of 22 animals corresponding to the Caribbean Region, were captured and sampled in the Provinces of Córdoba (n = 12), Sucre (n = 6), Bolívar (n = 2), and Magdalena (n = 2). In turn, deer of the Province of Córdoba were captured and sampled in the municipalities of Montería and San Pelayo, while deer of the Provinces of Sucre, Bolívar, and Magdalena were captured and sampled in the municipalities of Sincelejo, Magangué, and Santa Ana, respectively. On the other hand, the 22 animals of the Orinoquía region were captured and sampled in the municipality of Saravena, Province of Arauca.

Serums were obtained using a manual centrifuge, at 3,000 rpm and were transported in liquid nitrogen to the laboratory where they were stored at -80°C until analysis.

Enzyme-linked immunosorbent assay (ELISA) testing. Serum samples were analyzed using a commercial indirect pre-absorbed ELISA test, according to instructions of the manufacturer (Cattletype® MAP, Qiagen Leipzig GmbH, Leipzig, Germany). The results were considered as valid, if the mean value (MV) of the measured OD value for the positive control (PC) was ≥ 0.7 and if the MV of the measured OD value for the negative control (NC) was ≤ 0.2. An animal was considered ELISA-positive at a sample-to-positive ratio (S/P%) of ≥ 0.4, as recommended by the manufacturer.

Data analysis. All the information generated during the study was entered into Excel spreadsheets (Microsoft Corp., Redmond, WA, USA) and then exported to Stata 14.0 (StataCorp, 2017, Texas, USA) for statistical analysis. Descriptive statistics were calculated for all variables (i.e. capture location, sex, age group), and the association between these variables and the serological result ($p<0.05$) was explored, using Pearson's *chi*-square test.

RESULTS

The 54.5% (24/44) of sampled deer were females, while 45.5% (20/44) were males. The 50% (22/44) of the animals tested positive for the presence of anti-MAP antibodies. Ten out of 22 of these animals were females, while 12 were males. The 18.2% (4/22) of the animals were detected as seropositive in the Caribbean region, while 81.8% (18/22) were detected as seropositive in the Orinoquía region. The statistical analysis found no associations between the study variables and the result obtained by ELISA. The characteristics of the sampled population, in terms of the serological result obtained, are presented in table 1.

Table 1. ELISA-test results for the detection of anti-*Mycobacterium avium* subsp. *paratuberculosis* antibodies in wild deer (*Mazama rufina* and *Odocoileus virginianus*) from Colombia.

Region	Province	Municipality	ELISA results		
			+	-	Total
Caribbean	Cordoba	Montería	4	4	8
		San Pelayo	0	4	4
	Sucre	Sincelejo	0	6	6
	Bolivar	Magangué	0	2	2
	Magdalena	Santa Ana	0	2	2
Orinoquia	Arauca	Saravena	18	4	22
Total			22	22	44

DISCUSSION

The present study aimed to determine the presence of anti-MAP antibodies detected by ELISA in free-ranging deer captured in two different natural regions of Colombia. To the authors´ knowledge, this is the first study exploring MAP infection in wild animals in the country. The seropositivity found (50%) could be explained by the method of sampling, which was done by convenience and does not allow to obtain a representative sample of the population. It is also important to note that ruminants can develop a disease without an antibody response or an inconsistent response against MAP (13). Such particularities could have affected the frequency of MAP infection reported here, due

to false negative results. In any case, high seropositivity rates in animals use for human consumption is a concern in public health to the higher probabilities of infected animals could transmit the bacteria to human food chain through MAP-contaminated meat (14). Even more so when in Colombia the hunting meat it is not supervised by the sanitary authority, the national institute for the surveillance of drugs and foods (INVIMA; by its name in Spanish, instituto nacional para la vigilancia de medicamentos y alimentos).

The most plausible hypothesis to explain the presence of anti-MAP antibodies in deer in Colombia, is the residual ingestion of the bacteria from a previously-contaminated environment with feces from infected domestic or wild ruminants, and even non-ruminant animals that share that habitat, as reported by other studies (5,6,15). In the region of study it is very common that domestic ruminants share pastures and water resources with wildlife species. Under certain conditions the environment plays an important role in the transmission of MAP within species that can contribute to the survival of the bacteria up to one year in the soil or even longer in water (16). On the other hand, the transmission of bacteria of the genus *Mycobacterium* between livestock and wild species included a free-ranging deer have been suggested by several authors and can be explained by the fact that share food and water sources (15,17) but may also infect wild animals and occasionally humans. Its principal infective agent is Mycobacterium bovis. The present study was undertaken to examine the possible involvement of wildlife in the M. bovis life cycle in the Pantanal and to assess their potential role as a wild animal reservoir for this disease. DNA samples were obtained from 14 wild animals, namely 4 pampas deer (Ozotoceros bezoarticus. However, the dynamics associated with the bovine population in the area cannot be explored, since there are no MAP/PTB reports in the area of interest to date (11).

According to the Colombian agricultural institute (ICA; by its name in Spanish, instituto colombiano agropecuario) —public entity responsible for the prevention, surveillance, and control of health, biological, and chemical risks for animal and plant species, the regions of study gathers 30 and 21% of the bovine population of Colombia, respectively. This distribution shows the important participation of these two regions in the national cattle production and the possible impact they can have on the ecosystems where they are developed. The extensive livestock system in these two natural regions of Colombia is characterized by having a low cattle density (approximately 1.5-1.8 animals/Ha). In addition, cattle freely grazing on natural plains with no fences are a very common practice, so, the direct contact with wild deer or their feces is quite possible.

These findings support the need for further studies using direct diagnostic techniques (bacteriological and molecular), and investigative approaches that allow the definition of links in the dynamics of infection between wild and domestic mammals in Colombia, considering specific risk factors at population-level in the extensive systems of Colombian cattle. It is also necessary to clarify the role of deer in the epidemiology of MAP, for this it is necessary to use tools such as genotyping that allow tracking sources and transmission patterns. The level of genetic diversity obtained by combining genotyping techniques (i.e. MIRU-VNTR, MLSSR, SNPs) is a promising pathway for molecular epidemiology investigations of this pathogen (18).

Conflict of interest

The authors are not aware of any financial or personal relationships with other people or organizations that could inappropriately influence the work reported in this paper.

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