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Original

Seroprevalence to *Mycobacterium avium* subsp. *paratuberculosis* in the colombian creole cattle Romosinuano and costeño con cuernos

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ABSTRACT

Objective. Determine the seroprevalence and epidemiological factors associated with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in two Creole cattle breeds of the Turipaná research center - AGROSAVIA. **Materials and methods.** A cross-sectional epidemiological study was conducted,a total of 848 Creole bovine animals were sampled, 403 Romosinuano and 445 Costeño Con Cuernos (CCC); for the serological diagnosis of antibodies, the Elisa Indirect test was used with the commercial kit Parachek®2 by Prionics. The variables sex, age, breed and herd type were the epidemiological factors evaluated and correlated with the presence of antibodies against MAP; the statistical association was established using the Odds Ratio and a multivariate logistic regression model, employing a significance level with $p<0.05$. **Results**. The general seroprevalence of the Creole cattle to MAP was 2.35% (95% CI, 1.34-3.38); however, in the Romosinuano it was 0.74% and in the CCC it was 3.82%, being this difference statistically significant ($p=0.003$). Furthermore, employing a univariate way analysis, females and animals older than one year of age were more affected. The multivariate analysis identified the breed and sex variables as epidemiological factors. **Conclusions**. In the Creole breeds of the AGROSAVIA-Turipaná research center, MAP seroprevalence was low; however, in the Costeño Con Cuernos breed, the risk of contracting the disease is six times higher than in the Romosinuano breed. Moreover, it was shown that females have a higher risk of acquiring the disease.

Keywords: Agrosavia; serological diagnosis; Johne's disease; enteropathy; epidemiology; risk factors (*Sources: DeCS, FAO*).

RESUMEN

Objetivo. Determinar la seroprevalencia y los factores epidemiológicos asociados a *Mycobacterium avium* subsp *Paratuberculosis* (MAP) en dos razas de bovinos criollos del centro de investigación AGROSAVIA-Turipaná. **Materiales y métodos**. Se realizó un estudio epidemiológico de corte

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transversal. Fueron muestreados 848 bovinos criollos, 403 Romosinuanos y 445 Costeño Con Cuernos (CCC); para el diagnóstico serológico de anticuerpos se utilizó la prueba Elisa Indirecta mediante el kit comercial Parachek® de Prionics. Las variables sexo, edad, raza y tipo de hato fueron los factores epidemiológicos evaluados y correlacionados con la presencia de anticuerpos contra MAP; la asociación estadística fue determinada mediante Odds Ratio y con un modelo multivariado de regresión logística, utilizando un nivel de significancia con p<0.05. **Resultados**. La seroprevalencia general de los bovinos criollos a MAP fue de 2.35% (IC 95%, 1.34–3.38); sin embargo, en los Romosinuano fue de 0.74% y en los CCC fue de 3.82%, siendo las diferencias estadísticamente significativas (valor p=0.003). También, empleando un análisis univariado, fueron más afectados las hembras y los animales mayores a un año. El análisis multivariado identificó como factores epidemiológicos las variables raza y sexo. **Conclusiones**. En las razas criollas del centro de investigación AGROSAVIA-Turipaná, la seroprevalencia a MAP fue baja; sin embargo, en la raza CCC el riesgo de contraer la enfermedad es seis veces mayor con relación a la raza Romosinuano. Más aún, se pudo evidenciar que las hembras tienen mayor riesgo de adquirir la enfermedad.

Palabras clave: Agrosavia; diagnóstico serológico; enfermedad de Johne; enteropatía; epidemiología; factores de riesgo (*Fuentes: DeCS, FAO*).

INTRODUCTION

Paratuberculosis or Johne's disease is characterized by the chronic granulomatous inflammation of the digestive tract that mainly affects domestic ruminants. Its clinical manifestation is an enteropathy with protein loss that causes diarrhea, emaciation or wasting, hypoproteinemia and in some cases, death (1). The causative agent is *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (2). In young bovines, the early transmission occurs through the oral-fecal route by food and milk ingestion and contact with contaminated areas (3).

MAP infection occurs in the first months of the animal's life; after a long incubation period, the clinical signs appear when the animal is between two and five years of age (4).

Bovine paratuberculosis causes substantial economic losses due to the negative effects on animal health, production, and reproduction (5). It is included in the list of mandatory declaration of infectious diseases and must be notified to the World Organization for Animal Health - OIE (6). For adequate control, the infection status of the herds, the routes of transmission and the different sources of infection must be identified (3).

Among the tests for the detection of antibodies against MAP, the ELISA technique is the most commonly available and widely used, although it has low sensitivity, especially in the early stages of the disease (7). In relation to the diagnosis of the causative agent, it shows a long waiting stage due to a prolonged incubation period and contamination risks (8).

In South America and the Caribbean, it has been estimated that the general prevalence of the disease in cattle is 16.9%, and at the herd level, it reaches 75.8% (9). In Colombia, bovine paratuberculosis was first diagnosed in 1924; years later, other cases were reported in the department of Meta by Instituto Colombiano Agropecuario (ICA) (10).

Although the circulation of MAP in Colombia has been confirmed for many years, the studies carried out are insufficient to show its magnitude. However, the number of original investigations carried out in the last decade suggests a growing interest in this microorganism in the country (11).

In Colombia, there are several germplasm banks of Creole cattle intended for the conservation of genetic diversity; however, there are few published studies of MAP prevalence in these repositories of Colombian Creole cattle (12); accordingly, the aim of this study was to establish the seroprevalence and epidemiological factors associated with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in the Creole cattle breeds Romosinuano and Costeño Con Cuernos of the research center Turipaná-AGROSAVIA.

MATERIALS AND METHODS

Study site. The study was carried out at the Turipaná research center of AGROSAVIA; the center is located between latitudes 8º51'50" N and 8º49'40" N, and longitudes -75º40'30" W and -75º46'10" W, in the municipality of Cereté, department of Córdoba, Colombia. The region is classified as a tropical dry forest with an

altitude of 14 meters above sea level, an average temperature of 27.5ºC, 81% relative humidity, and an annual average rainfall of 1340 mm. The region has a period of drought and another of rains that starts in April and ends in November.

Study type and sample size. A cross-sectional study was carried out where all the individuals of the Romosinuano (403 animals) and Costeño Con Cuernos (445 animals) breeds of the Turipaná research center were evaluated during the months of May to October 2016. The age of the sampled animals ranged from one month to 15 years as follows: 160 animals under six months of age, 42 between six and 11 months of age, and 616 from one year onwards.

Sample collection, processing, and analysis. After disinfection of the area, 5 ml of blood was collected from the coccygeal vein of each animal in vacutainer-type tubes without anticoagulant. The samples were identified and stored at a temperature of 4°C for approximately 4 hours until serum was obtained. For this, the samples were centrifuged at 3500 rpm for 5 minutes, deposited in vials and stored at -20°C until analysis in the laboratory of the Tibaitatá research center of AGROSAVIA, in the department of Cundinamarca, Colombia. The analysis was performed employing an INDIRECT ELISA test using the commercial diagnostic kit Parachek®2 Prionics AG., according to the manufacturer's recommendations.

Statistical analysis. The seroprevalence study was accompanied by an epidemiological survey aimed at determining risk factors that could be related to the pathology under study; sex, breed (Romosinuano and CCC), age (under one year of age and over one year of age) and herd (germplasm bank and genetic breeding program) were the factors assessed. These factors were associated univariately with the diagnostic results; Odds Ratio (OR) was established as an effect measure and confirmed by the Chi-square statistic or Fisher's exact test with a significance level of 0.05. Finally, a multivariate logistic regression model was constructed to explain the correlation between the factors and the seroprevalence of paratuberculosis; the variables included in the model were preselected through the univariate analysis, including those that showed $p<0.2$. The data were analyzed with the EpiInfo 7.2.1.0 software.

RESULTS

Of the 848 samples analyzed, MAP antibodies were found in 20 animals, indicating an apparent seroprevalence of 2.35% (95% CI, 1.34-3.38) for the Creole bovine breeds of the Turipaná research center.

Univariate analysis. Table 1 shows the univariate analysis of the variables studied, observing that only sex and breed showed a statistical association with MAP seroprevalence.

Multivariate analysis. Considering the logistic regression model (Table 2), the sex and breed variables maintained their statistical significance; therefore, animals of the CCC breed are 5.6 times more susceptible to being seropositive to MAP, and Creole bovine females are 4.7 times more positive than Creole males.

Table 1. Univariate analysis of epidemiological factors associated with MAP seroprevalence in Creole cattle of the Turipaná - AGROSAVIA research center.

Variables	Categories	n+	n-	Seroprevalence	p-value	Odds ratio	Confidence interval	
							Lower	Upper
Breed	Romosinuano	3	400	0.74%	0.003	5.296	1.540	18.201
	Costeño con Cuernos	17	428	3.82%				
Sex	Male	2	271	0.73%	0.032	4.378	1.008	19.002
	Female	18	557	3.13%				
Age	≤ 1 year	2	230	0.86%	0.078	3.461	0.797	15.041
	> 1 year	18	598	2.92%				
Herd	Genetic breeding program	1	81	1.22%	0.475	2.060	0.272	15.593
	Germplasm bank	19	747	2.48%				

Table 2. Logistic regression model for MAP in the AGROSAVIA - Turipaná Creole cattle herd.

Variables	Categories	p-value	Odds ratio	Confidence interval	
				Lower	Upper
Sex	Male Female	0.037	4.753	1.091	20.710
Breed	Romosinuano Costeño con Cuernos	0.006	5.608	1.627	19.328

DISCUSSION

The seroprevalence found in this study (2.35%) in Creole bovine breeds of the Turipaná research center was similar to the one found in dairy herds of the department of Antioquia (2%) using the same ELISA kit (Parachek®2 Prionics AG.) (13). On the other hand, Vélez et al (12), in a study conducted in the Creole cattle breed Blanco Orejinegro did not find seroprevalence for this disease using the ELISA technique.

In the department of Córdoba, in 2010, a seroprevalence of 25% was detected in dairy cows (14). Recently, Caraballo et al (15) determined the presence of MAP in bovine feces in the department of Sucre, visualizing *Mycobacterium* in 21.15% of the total samples analyzed; another study in beef cattle in the municipality of Caucasia in the department of Antioquia showed that 33.8% (n=151) of the cattle studied were seropositive to MAP (13); in dairy farming in the department of Nariño, 8% of the animals (n=958) were seropositive (16). These seroprevalences were higher than those found in this study, however, none exceeds the values found in 2006 in dual-purpose livestock in Venezuela, where 72.1% (n=240) of the cattle were seroprevalent (17).

Variable breed. Some studies show a low prevalence of MAP in Creole breeds. For example, 1.45% seropositivity (n=207) was established in the Limonero breed (8) and 0% in Blanco Orejinegro animals (12); in this last study, seropositivity was observed in animals with crosses with Brahman genetics. Although in the current study, the prevalence of MAP was low in both breeds, given the similar population, management and feeding conditions, a lower susceptibility of the Romosinuano breed was evidenced, which could be explained by possible genetic differences. The animals of the CCC breed were 5.3 OR more affected in relation to the Romosinuano. However, since it is the first study of paratuberculosis carried out in these two breeds, further work is required to understand the higher susceptibility of the CCC breed.

Variable sex. Univariate analysis of this variable determined that females were 4.37 OR more affected than males. The highest susceptibility of females could be attributed to immunological alterations and stress caused during birth and lactation (18).

Variable age. The highest prevalence was found in animals older than one year, with 2.92% seropositivity; however, no statistical difference was found between the two age groups and MAP seropositivity. Similar results were observed in cattle studied in Caucasia, located in the northeast of Antioquia (12), and in the south of Nariño (16).

When paratuberculosis is studied considering the age variable, the specificity of the trial becomes a challenge; in milk cattle, it has been shown that the time elapsed from infection, usually in a calf, to the clinical disease, varies from one to 12 years (19); additionally, serodiagnosis is usually negative in animals under one year old because during the early stages of the disease, the cell-mediated immune response predominates, while the serum antibody response is minimal or absent (20).

Considering the above, it can be considered that serological tests such as ELISA performed only once, are not able to identify the subclinical disease in all animals; therefore, it is suggested that these tests should be performed periodically to detect infected animals in the herd before their clinical manifestation.

Herd variable. The management and feeding conditions were similar between the herds belonging to the Germplasm Bank and the Genetic Breeding Program; the difference between the two herds is related to the purpose. The Germplasm Bank is a repository for the conservation of genetic diversity, and the Genetic Improvement Program is for strategic use, i.e., to utilize the advantages of the breed. Fisher's exact test showed no statistical association between the two herds; however, 95% (19/20) of the positive animals were found in the Germplasm Bank, a situation that could be influenced by grouping 90% of the animals in the study, allowing a higher transmission. Another study identified that the size of the herd is a significant factor for the propagation and prevalence of MAP

(21), so internal biosecurity measures should be implemented to reduce the exposure of the disease according to the characteristics of each herd (22).

Serological tests are widely used to estimate MAP prevalence in herds, however, the low sensitivity of these tests can give a substantial amount of false negative results (23); the test used in this study reports 99% specificity and up to 80% sensitivity in cattle before the appearance of the clinical signs. Due to the low prevalence found in this study, the number of false negatives would not significantly increase the true prevalence of the herd; new studies are projected mainly in animals that were less than one year old.

It should be noted that, in the logistic regression model, the sex and breed variables maintained their statistical significance.

This is the first study of bovine paratuberculosis that is carried out in Colombia for the Romosinuano and CCC Creole breeds. In conclusion, although a relatively low seroprevalence for MAP was reported in the two Creole breeds of the Turipaná Research Center, it is necessary to implement control and prevention measures to reduce the risk factors identified and associated with this disease.

Conflicts of interest

The authors declare that there are no conflicts of interest in relation to this article.

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