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ORIGINAL ARTICLE

Retrospective seroepidemiological study of small ruminant lentivirus, paratuberculosis and brucellosis in goats from Mexico, based on multiplex assay

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ABSTRACT. Mexican goat production systems face infection risks from *Brucella melitensis*, small ruminant lentivirus (SRLv) and *Mycobacterium avium* subsp. *paratuberculosis* (MAP); agents that cause great economic losses and directly affect public health (brucellosis and paratuberculosis [PTb]). Currently, there are no diagnostic tests applicable at large scale nor epidemiological information regarding the seroprevalence for these infectious diseases. For this study, a multiplex antibody assay (Luminex®) was used to retrospectively study the seroprevalence of brucellosis, SRLv, and PTb in the sera of 983 goats from nine Mexican states. Sera were obtained between 2014 and 2019. Antibodies against all three infectious diseases were detected in almost all tested samples. The estimated seroprevalence values ranged from 37% to 78% for brucellosis, 21% to 65% for SRLv, and 0% to 13% for PTb. The multiplex assay (Luminex®) is a simple, accessible, efficient, and cost-effective seroprevalence monitoring tool for brucellosis, SRLv, and PTb, and can be used as a large-scale approach.

Keywords: Caprine, diagnostic, antibodies, Luminex®, serological.

INTRODUCTION

Goat production in Mexico is traditionally carried out in economically poor regions, particularly arid and semi-arid regions. This activity represents one of the main sources of income for the population in these regions (Secretaría de Agricultura y Desarrollo Rural, 2016). The majority of goat herds are destined for self-sustenance and are characterized by low levels of technology implementation. For example, there is a lack of health records, grouping according to age or production stage, and it is common practice to keep them in shared communal pasturelands (Cuellar *et al*., 2012). The latter favors contact between animals from different herds and other animal species (e.g., cattle and sheep), which increases the risk of infection and compromises the control of relevant infectious diseases such as brucellosis, small ruminant lentivirus (SRLv), and paratuberculosis (PTb).

Since 2010, the National Council for Animal Health (CONASA, Consejo Técnico Consultivo Nacional de Sanidad Animal) has designated brucellosis and PTb, which are potentially zoonotic diseases, and small ruminant lentivirus and mycoplasmosis, as relevant diseases (CONASA, 2011).

Brucella melitensis is the main cause of brucellosis in goats (Garin -Bastuji *et al*., 1998; Aguilar *et al*., 2011; Díaz-Aparicio, 2013), characterized by abortions towards the end of gestation and occasionally orchitis or epididymitis (Chand *et al*., 2002). This agent, *B. melitensis*, is considered to be of public health importance as it is the most pathogenic *Brucella* spp. in humans (Méndez *et al*., 2015). In Mexico, two diagnostic tests are commonly used and recommended for the determination of caprine brucellosis: agglutination tests using *B. abortus* strain 1119-3, and complement fixation as a confirmatory test (Norma Oficial Mexicana, 1995). Furthermore, a native hapten (NH) from *B. melitensis* has been developed to differentiate vaccinated from naturally infected animals, with the aim of targeting vaccination and other control strategies in different herds (Moreno, *et al*., 1987; Zygmunt *et al*., 1988; Díaz-Aparicio *et al*., 1996).

Small ruminant lentivirus infects sheep and goats worldwide, causing multisystemic chronic and progressive disease, characterized by pneumonia, encephalitis, arthritis, and mastitis (Minguijón *et al*., 2015). In Mexico, SRLv was officially recognized in goats in the 1990s and sheep in 2016

(Mendiola *et al*., 2019). The main SRLv diagnostic techniques are Agar Gel Immunodiffusion (AGID) and ELISA (Fry *et al*., 2008; Martínez *et al*., 2012; OIE, 2017a), with PCR considered a complementary technique (De Andrés *et al*., 2005; OIE, 2017a).

Paratuberculosis, also known as Johne's disease, is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (Robbe, 2011; Fiorentino *et al*., 2012). The clinical disease is characterized by chronic enteritis, decreased milk production, and progressive weight loss, which causes emaciation and death in adult animals (Estévez-Denaives *et al*., 2007; Kheirandish, *et al*., 2009). The most reliable method for the diagnosis of MAP is fecal culture (OIE, 2017b, 2021), but it is hardly used due to the prolonged incubation periods required (> 6 weeks) (Estévez-Denaives *et al*., 2007). ELISA has been proposed as an alternative diagnostic test for seropositive animals (Stabel *et al*., 2009).

Based on the importance of the above-mentioned diseases for Mexican goat production systems, the aim of this study was to conduct a retrospective seroepidemiological study to estimate the prevalence of brucellosis, SRLv, and PTb in goats from different regions of Mexico using a multiplex assay (Luminex®). This test is highly efficient because it reduces the detection time and required sample volumes. Additionally, it's a reliable, reproducible, sensitive, and specific test with high accuracy and precision indices (Ray *et al*., 2005; Elshal & McCoy, 2006; Anderson, 2011; Nájera-Rivera *et al*., 2023) and has been used in the diagnosis of other infectious diseases (Ravindran *et al*., 2010; Anderson *et al*., 2011; Rodriguez *et al*., 2023).

MATERIALS AND METHODS

A total of 983 goat sera obtained from the goat sera bank of the Faculty of Veterinary Medicine (UNAM) were analyzed. The samples correspond to convenience sampling conducted in nine different states of the Mexican Republic between 2014 and 2019. The samples were taken in a few municipalities per state at different times due to the availability of animals and not across the indicated years. Table 1 shows the number of samples analyzed by state and municipality.

To perform the triplex assay (Luminex® Corporation, Texas, USA), the protoplasmic antigen (PPA-3) (Allied Monitor Laboratory, Missouri, US) from MAP, native hapten (NH) from *B. melitensis*, and recombinant proteins p16 and gp38 from SRLv were used. PPA-3 corresponds to a bacterial lysate from the strain *M. avium*, which has been demonstrated

Table 1.
A total of 983 samples obtained from 2014 to 2019 through convenience sampling were analyzed and categorized by state and municipality.

State	Municipality	Number of samples per municipality	Total samples/State
Estado de México	Capultengo	32	48
	Ixtapaluca	16	
Baja California Sur	Comondú	37	37
Coahuila	Matamoros	43	76
	Viesca	33	
Guanajuato	Pénjamo	24	180
	Salamanca	43	
	Santa Cruz de Juventino Rosas	113	
Querétaro	Tequisquiapan	339	339
Sinaloa	Culiacán	81	81
Sonora	Cajeme	22	22
Tlaxcala	Altzayanca	102	102
Veracruz	Altotonga	67	98
	Coatepec	31	
TOTAL		983	983

to be immunogenic, with sensitivity values of up to 80% and specificity of 90–95% (Costanzo *et al.*, 2012). For SRLv, the genes encoding the matrix protein (P16) and transmembrane protein (gp38) from the SRLv genome subtype B were amplified and cloned into an expression vector (Hötzel & Cheevers, 2001; Vázquez Franco, 2011). Native hapten antigen, a 14.5 kDa polysaccharide, was obtained using a previously described method (Moreno *et al.*, 1987; Zygmunt *et al.*, 1988).

The four antigens used comprised a triplex diagnostic panel. The procedure was standardized and previously validated; its sensitivity was between 84.4% and 98.9%, and its specificity was between 95.9% and 98.4% (Nájera-Rivera *et al.*, 2023). In the case of SRLv, sera that reacted with both p16 and gp38 antigens were considered seropositive.

Statistical analysis

Seroprevalence was calculated through the detection of antibodies that recognize each of the antigens used, with a confidence interval of 95%. An imperfect test was performed using EpiR software in the open source R software (R Core Team, 2013) (Rosati *et al.*, 2004; Mark *et al.*, 2023). This statistical test uses the apparent prevalence, as well as the sensitivity and specificity of the diagnostic test to obtain an estimated prevalence value (Rogan & Gladen, 1978), whereas the confidence intervals of the estimated prevalence were obtained using the methodology proposed by Reiczigel *et al.* (2010).

RESULTS

Based on the serological multiplex assay, all the samples were positive for at least one of the diseases tested in this study. Antibodies against the agents studied were found in all sampled Mexican states. In general, brucellosis and SRLv showed a high estimated prevalence (37% and 21%, respectively). Meanwhile, the PTb-estimated seroprevalence was low in almost all states, with Guanajuato having the highest seroprevalence of 13%. The percentages of the estimated seroprevalence and confidence intervals for each state are shown in Table 2. Figure 1 shows the geolocation of the sampled municipalities.

DISCUSSION

This retrospective seroepidemiologic study was conducted in nine Mexican states using the multiplex Luminex® technique to estimate seroprevalences (from 2014 to 2019)

Table 2.
The estimated seroprevalence percentages and confidence intervals were calculated for brucellosis, SRLv, and PTb, by state. The highest estimated seroprevalence was observed in brucellosis and SRLv. Antibodies against these antigens were present in all Mexican states where the samples were collected.

State	Estimated seroprevalence by disease (intervals)		
	Brucellosis	SRLv	PTb
Estado de México	73% (58.53-84.56)	64% (48.83-76.79)	8% (1.06-21.63)
Baja California Sur	71% (53.43-83.58)	21% (9.70-37.01)	5% (-0.96-20.62)
Coahuila	76% (64.09-84.50)	44% (32.42-55.44)	0% (-2.65-8.58)
Guanajuato	67% (59.05-73.60)	54% (46.38-61.65)	13% (8.04-20.20)
Querétaro	55% (49.39-60.60)	57% (51.65-62.75)	2% (-0.54-4.97)
Sinaloa	50% (38.23-60.90)	41% (30.13-52.28)	4% (-0.21-13.54)
Sonora	78% (55.93-91.43)	65% (43.10-82.59)	6% (-1.27-28.21)
Tlaxcala	37% (27.94-47.78)	27% (18.53-36.60)	0% (-2.43-7.03)
Veracruz	74% (63.81-82.19)	44% (34.12-54.53)	2% (-1.66-9.06)
Total	60% (57.08-63.58)	49% (45.59-52.20)	4% (2.60-6.42)

SRLv, Small ruminant lentivirus; PTb, Paratuberculosis.

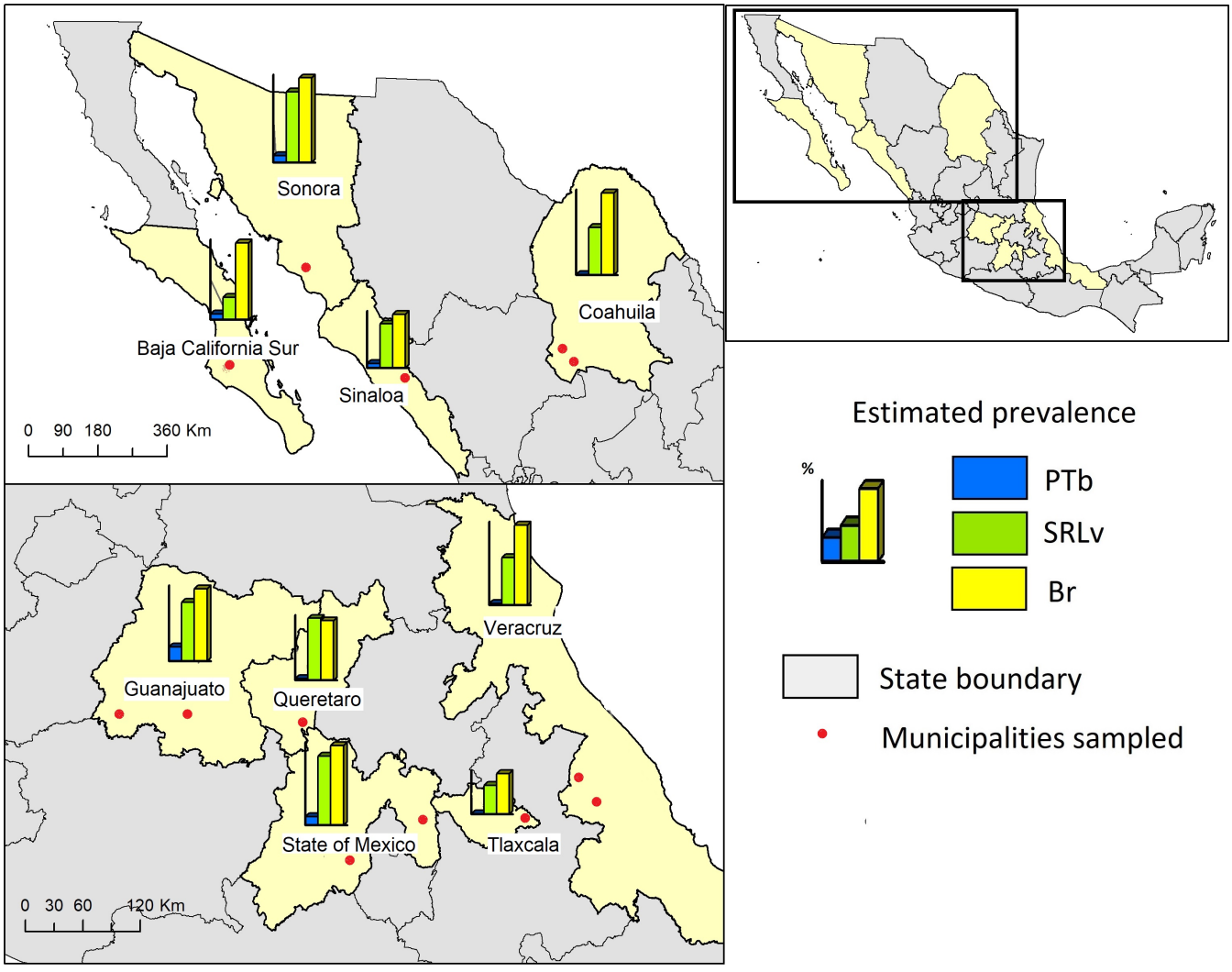

Figure 1.
Map with geolocation of sampled states and municipalities. The estimated seroprevalence and geolocation for brucellosis, SRLv, and PTb are shown in histograms by state. Brucellosis and SRLv had the highest estimated seroprevalence among all sampled states.

for brucellosis, small ruminant lentivirus, and paratuberculosis, which are considered the most relevant infectious diseases in the goat industry in Mexico (Palomares *et al.*, 2021).

The results of this study showed that the three identified diseases had a recurring presence throughout the country. Among the analyzed states, brucellosis (60%) and SRLv (49%) showed the highest total seroprevalence. Despite the detection of antibodies against MAP in most states, the total estimated seroprevalence was low (4%).

The seroprevalence of brucellosis was higher than the national seroprevalence of 0.05% reported by the National Health Service (SENASICA) from the Secretariat of Agriculture (SADER) in 2018 (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria, 2022). However, this official prevalence report was generated using a non-randomized sampling approach, mostly based on voluntary farmer participation; therefore, a possible sampling bias may underestimate the prevalence of brucellosis in the country. Thus, this sampling approach cannot be used as a valid reference for caprine brucellosis in Mexico.

In our study, brucellosis was present in every state where sampling was carried out and had the highest number of seropositive animals among the three evaluated diseases. Owing to the higher sensitivity of the multiplex assay, it is possible that we identified a higher number of seropositive animals than other tests used to monitor this disease (Elshal & McCoy, 2006; Anderson, 2011).

Of the three diseases evaluated in this study, brucellosis was the only one that had a proper and official routine diagnosis, for which vaccination is mandatory (Norma Oficial Mexicana, 1995). Nevertheless, an important problem when vaccinating goats against brucellosis is that no information

is regularly recorded, that is, animal ID, dose used, or date of immunization. The high prevalence of seropositive samples in this study is due to natural infections caused by field strains and not due to vaccination, since the NH antigen does not detect vaccine antibodies (Díaz-Aparicio *et al*., 1994; Díaz-Aparicio *et al*., 1996).

In Mexico, the predominant SRLv is genotype B1 (Ramírez *et al*., 2011), and the proteins encoded by the lentivirus *gag* gene (capsid and matrix) are used in serological tests that are highly sensitive for the detection of antibodies for prolonged periods of time and early stages of the infection (Grego *et al*., 2005). Additionally, the combined use of these recombinant proteins in ELISA-type tests generates a diagnostic efficiency greater than that obtained individually (Rosati *et al*., 2004). In the present study, the estimated seroprevalence of SRLv was 49% in all the studied Mexican states, comparable to the 53.9% reported in 2021 in six states using ELISA (De la Luz *et al*., 2021). Moreover, the prevalence found in four of the studied states was greater than 50% (Sonora 64%, Estado de México 63%, Querétaro 56%, and Guanajuato 53%), which is similar to previous reports for this state (De la Luz *et al*., 2021). Even in states where we found a lower prevalence, these were still considered high (Tlaxcala 27%, and Baja California Sur 21%). This situation can be explained by the fact that SRLv is not part of any program to control this disease in Mexico. Moreover, the high seroprevalence estimated for SRLv in this study could be due to the high sensitivity of the Luminex ® technique, in which the detection of seropositive animals was determined using two recombinant SRLv proteins of genotype B1, giving the technique a higher probability of detecting true positives. It is also possible that the infection is increasing in the country's herds, since in the 80s, it was estimated that 27% of the animals were seropositive (Nazara *et al*., 1985).

Caprine PTb is an endemic disease widely disseminated in Latin America. However, in Mexico, few studies have determined its seroprevalence in certain regions or states (Mejía *et al*., 2015; Espeschit *et al*., 2017). The highest PTb seroprevalence in this study was 13% in Guanajuato. A study conducted by Favila *et al*. (2009) also found high seroprevalence in the same state (22%). This could be related to the intensive milk production system used, which favors the spread of infection among animals. Conversely, a low PTb prevalence was reported for Guanajuato using a different test, AGID (Meza *et al*., 2019), which is less sensitive than Luminex® used in this study.

The states with the lowest PTb prevalence in our study (< 2%) were Coahuila, Tlaxcala, Veracruz, and Querétaro, comparable to those previously reported for Veracruz (0.6%) (Villagómez *et al*., 2012), but lower than those reported for Coahuila (15%) (Toledo *et al*., 2010). In this study, we observed a total seroprevalence of 4%, with no state showing seroprevalence above 8%, except for Guanajuato. This finding is consistent with that reported by Morales *et al*. (2020), who reported a prevalence of 7% in Sonora. However, in other studies in which ELISA was used, the seroprevalence was higher (Fávila *et al*., 2009, 2010; Toledo *et al*., 2010; Gallaga *et al*., 2017).

These differences may be due to the use of techniques with different sensitivities and specificities, or to a low seroconversion of the animals because the long incubation periods of this infectious agent make its early detection difficult in truly infected animals (Ramírez *et al*., 2011). Therefore, a comparison of the results with previous reports should be performed with caution. Nevertheless, our data indicate that this disease is widespread in Mexico. Adequate control of PTb in the clinical and subclinical stages in animals (Espeschit *et al*., 2017) is of public health importance because it has been associated with Crohn's disease in humans (Sutton *et al*., 2000; Sechi *et al*., 2005; Hermon-Taylor, 2009).

In the present study, it was shown that the use of a multiple diagnostic test, such as Luminex®, with high sensitivity and specificity parameters is functional for large-scale diagnosis. Furthermore, the test is easy, affordable, and allows efficient sample usage.

The sample size used in this study was minimal because they came from a serum bank, obtained by convenience; therefore, the randomization condition that is essential for obtaining reliable epidemiological values was not fully met (Pérez *et al*., 2017). Likewise, the comparison of the data from this study with other reports has the limitation of not knowing the sensitivity and specificity values of each test. However, the estimated prevalence implies that brucellosis, small ruminant lentivirus, and paratuberculosis are present in Mexico. Therefore, it is important to carry out serological monitoring to confirm the current status of these diseases in different regions and herds of the country and thus implement the most effective epidemiological measures to preserve animal and human health.

It is worth noting that the samples tested in our study were taken between 2014-2019, and during this period, several control measures and programs may have been put in place to control the diseases reported here. For example, we described an estimated prevalence of brucellosis in Sonora of 78% in samples collected in 2015, when an eradication program to control the disease was in place. Currently, brucellosis is considered to be eradicated in the State of Sonora (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria, 2022). Therefore, the present report represents an excellent reference for evaluating the success and effectiveness of control and eradication programs implemented during or after the study period.

CONCLUSION

This study showed that brucellosis, small ruminant lentivirus, and paratuberculosis are common in goat production systems in Mexico. Furthermore, we demonstrated that Luminex® technology is suitable for detecting antibodies against viral and bacterial antigens of high or low prevalence diseases in goat herds.

It is important to continue to monitor the status of these diseases to establish the most viable prevention, control, and eradication measures, according to the region and production system, particularly in the case of brucellosis and paratuberculosis, owing to their implications for public health.

DECLARATIONS

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Author contributions

ADRC: data curation, formal analysis, investigation, methodology, supervision, validation, writing of the original draft, writing review, and editing. CMC: data curation, formal analysis, and investigation. HDNR: data curation, formal analysis, investigation, validation, writing of the original draft, writing review, and editing. EDA: conceptualization, resources, supervision, writing review, and editing. ADP: conceptualization, resources, supervision, writing review, and editing. HRA: conceptualization, resources, supervision, writing review, and editing. ORC: formal analysis and visualization. AZG: formal analysis and visualization. EHL: resources, supervision, writing review, and editing. LCM: conceptualization, funding acquisition, project administration, resources, supervision, writing of the original draft, writing review, and editing.

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