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Chapter

Comparative Field Trial Effect of *Brucella* spp. Vaccines on Seroconversion in Goats and Their Possible Implications to Control Programs

Baldomero Molina-Sánchez, David I. Martínez-Herrera, Violeta T. Pardío-Sedas, Ricardo Flores-Castro, José F. Morales-Álvarez and José A. Villagómez-Cortés

Abstract

The aim of this study was to determine the seroprevalence of *Brucella* spp. in a goat flock and the seroconversion of three groups of animals vaccinated with Rev-1 (*Brucella melitensis*), RB51, and RB51-SOD (*Brucella abortus*) to estimate the level of protection conferred on susceptible females. Seventy-two animals were used by group. Goats were older than 3 months, seronegative to brucellosis, not vaccinated previously, and kept within positive flocks. Vaccinated animals received 2 mL of product subcutaneously in the neck region. The first block was injected with Rev-1; the second received RB51, and the third group was injected with RB51-SOD. Follow-up sampling was performed at 30, 60, 90, and 365 days post-vaccination. The general prevalence of brucellosis for the three groups was 1.2% (95%CI:0.5–2.7). The seroconversion rate by day 30 after vaccination was 77.7% (95%CI:61.9–88.2) for goats vaccinated with Rev-1. At 365 days post vaccination, the percentage of seropositive goats declined to 13.8% (95%CI:6.0–28.6). At day 365 after vaccination, 2.7% (95%CI:0.4–14.1) and 5.5% (95%CI:1.5–18.1) of animals vaccinated with RB51 and RB51-SOD, respectively, became positive. Results show that the seroconversion induced by *Brucella abortus* RB51 and RB51-SOD vaccines is lower than that by *Brucella melitensis* Rev-1.

Keywords: *Brucella*, vaccine, seroprevalence, seroconversion, goat

1. Introduction

The brucellosis is a highly contagious disease and one of the zoonoses worldwide; most importantly, it is caused by bacteria of the genus *Brucella* [1]. This has been classified by the World Health Organization (WHO) as one of the “top 10” neglected zoonoses, a group of diseases that are simultaneously ongoing threats to human health and a source of perpetuation for poverty [2]. The importance of the disease is enormous but remains under-prioritized worldwide, especially among

the pastoralists and small-scale livestock farmers. The humans can be infected by ingestion of food products such as unpasteurized milk and their derivative products contaminated with the pathogen or by direct interaction with an infected animal or by aerosol inhalation [1, 3].

In small ruminants, the brucellosis is caused by *B. melitensis* [4, 5], the most important agent that induces the disease in humans [6, 7]. The disease often occurs in cattle, sheep, and goat production units; the latter is the most important given its potential role in conveying disease to human. Because brucellosis is a public health problem, its presence and disease control strategies implemented in domestic ruminants affect the occurrence of disease in humans [8, 9]. In small ruminants, the disease is clinically characterized by a decrease in milk production, abortion, loss of weight, fetal death, placental retention, weak offspring, and acute orchitis. In dairy animals, *Brucella* spp. replicates in the mammary gland and supra-mammary lymph nodes, and infected animals continually excrete the pathogen into milk throughout their lives [10, 11].

In underdeveloped countries, vaccination is the main tool used in the control of this disease [12, 13], in particular as a preventive measure in small ruminants, and is considered necessary given the economic and medical consequences of having brucellosis in animals and people infected [14]. The main indicator of brucellosis reduction in animals is a concomitant reduction of human cases [13, 15]. In endemic areas, intensive vaccination with *B. melitensis* Rev. 1 strain in adult and young females has been developed, being the most popular vaccine for the control of brucellosis in small ruminants. The use of a reduced dose rate decreases the presence of vaccine-associated undesirable events, such as postvaccine reactors to conventional tests, abortion, and milk shedding [16]. The vaccination is recommended prior to the first gestation between 3 and 7 months of age to avoid abortion in pregnant animals [17]. When used at a reduced dose, Rev. 1 has shown to protect goats for at least 5 years after vaccination [13, 15]. El Idrissi et al. show that after vaccination, the animals vaccinated with Rev. 1 became positive to rose bengal plate test (RBPT) and complement fixation test (CFT) at 2 weeks, reaching the highest number of seroconverted animals' highest level between 2 and 6 weeks. Thereafter, the percentage of seropositive ewes declined to zero at 14 weeks after challenge. More than 75% of animals were seroconverted 15 days after challenge inoculation [18]. The seroconversion of vaccine is the persistent serological reaction, especially when animals are vaccinated as adults. These persistent serological reactions are mainly against the antigenic O-chain of the lipopolysaccharide present in smooth *Brucella* [19]. Some strains may generate diagnostic interferences in serological test [19, 20], like vaccines containing *Brucella* LPS O antigens that are detected by traditional serodiagnostic tests for brucellosis [21]. It has been reported that the average time from inoculation to seroconversion may range from 2 to 3 weeks in *B. melitensis*-infected goats, from 2 to 4 weeks in *B. abortus*-infected goats, and 3 weeks for the majority of tests evaluated with goats infected with either *Brucella* species [5, 19].

In Mexico, the vaccine RB51 was approved since 1998 as the official one for use in cattle females. The strain has been evaluated in both goat and sheep under controlled conditions with good protection against the experimental challenge with *B. melitensis*, even though protection is lower than that obtained with the Rev. 1 vaccine. Under experimental conditions no abortion occurs. Also, no postvaccination antibodies can be detected by conventional serology. The same findings have been reported after mass goat vaccination with RB51 in Veracruz, Mexico [13, 15].

Nowadays, the homologous overexpression to induce a greater and more effective immune response for the improvement of protective immunity of the vaccines has been developed. This can be achieved by introducing a plasmid within the RB51

strain with the gene that encodes the antigen expressed, along with appropriate promoters. In mouse (*Balb/c*) it has been shown that the overexpression of Cu/Zn superoxide dismutase (SOD) induces the best protection facing the experimental infection by *B. abortus* indicating that the homologous overexpression can produce a better vaccine RB51 (RB51-SOD) with an equal or better protection than that induced by Rev. 1, against the infection with *B. melitensis* [14, 19, 20]. However, there are no reports in domestic animals on the seroconversion and the vaccine efficacy. Therefore, the aim of this study was to determine the prevalence of *Brucella melitensis* in a goat flock and the seroconversion in animals vaccinated with Rev. 1 *Brucella melitensis*, RB51, and RB51-SOD *Brucella abortus* strains to estimate the level of protection conferred on susceptible females.

2. Material and methods

2.1 Study design

A phase III field trial was performed from September to December 2016 in order to determine the seroprevalence and seroconversion of goat flocks positive to brucellosis in the Xaltepec community municipality of Perote, Veracruz, Mexico, and to evaluate the protection conferred by vaccines with Rev. 1 *Brucella melitensis*, RB51, and RB51-SOD *Brucella abortus* strains.

2.2 Experimental design

The experiment was performed in two stages. In the first one, 546 animals from 14 herds with similar management, grazing, feeding, and confinement conditions were used to determine the prevalence of goat brucellosis in Xaltepec. In the second stage, groups required for vaccine evaluation were integrated by randomly selecting animals negative to serological tests meeting the inclusion criteria. Positive animals remained in the herds under field conditions in order to function as a challenge for healthy and vaccinated animals.

Sample size was calculated using Win Episcope Version 2.0 for simple random sampling, considering the 0.52% prevalence in goats reported in Veracruz by Román-Ramírez et al. of [12], a confidence interval of 95%, and an error margin of 5%. Since each animal had an identification number on its metallic earring, females were randomly assigned to each group and subgroup. For each group, the minimal calculated sample was 72 animals; each group was integrated by a vaccinated subgroup (36) and a not vaccinated or control subgroup (36). Studied groups were integrated by goats older than 3 months, seronegative to brucellosis, and not vaccinated previously and kept within positive flocks. Animals were randomly split into three groups and kept together 8 months in the flock to maintain exposure to *Brucella* spp.

2.3 Vaccination of animals

Animals in each vaccinated group received 2 mL of vaccine subcutaneously applied in the neck region. The first group was injected with Rev. 1 (*Brucella melitensis*) strain with a concentration of $1-2 \times 10^9$ CFU/mL, the second received RB51 strain (*Brucella abortus*) 3×10^8 to 3×10^9 CFU/mL, and the third one was injected with RB51-SOD (*Brucella abortus*) with a concentration of 3×10^8 to 3×10^9 CFU/mL. Each group had a control subgroup of unvaccinated animals which received 2 mL of PSS by subcutaneous injection in the neck region.

2.4 Sample collection

Follow-up sampling was performed at 30, 60, 90, and 365 days post vaccination by blood sampling collected from the jugular vein in vacutainer tubes without anti-coagulant (BD Vacutate, Oxford, UK). Each tube was identified with the number in the animal earring. Tubes containing blood samples were placed in a tilt position about 2 hours at room temperature allowing the separation of serum from the blood package. Later, tubes were placed into coolers at 4°C and transported to the laboratory and then were centrifuged at 1000 × g 10 minutes at room temperature. Finally, the serum was stored in sterile vials at –20°C until analysis.

2.5 Serological testing

Serum samples were analyzed by series using the following tests: 3% RBPT as screening and simple radial immunodiffusion test (SRD) as confirmatory [5, 22].

RBPT was used as a screening test on the serum samples collected for the presence of *Brucella* agglutinins. Equal volumes of test serum and *B. abortus* antigen strain 1119-3 at 3% and pH of 3.6 (Aba Test Tarjeta 3%) (National Producer of Veterinary Biologics PRONABIVE) were added and mixed. This test has shown 98% sensitivity and 100% specificity. This test gives presumptive results.

SRD was used as a confirmatory test, and the antigen was used at a concentration of 1 mg/mL on agarose gel prepared with a glycine buffer solution and native hapten obtained from *B. melitensis* 16 M strain (produced at CENID Microbiology Animal, INIFAP). The test has shown 96% sensitivity and 80–100% specificity for the differential diagnosis between goats infected with *Brucella* spp. and those vaccinated with the Rev. 1 strain.

2.6 Analyses of data

Seroconversion produced during the observation period was calculated. Differences between groups and the significance of association were calculated by chi square (χ^2), and the degree of association was estimated using relative risk (RR) [23]. In those cases that frequency of positive animals to tests was 0.0%, confidence interval was calculated according to Campbell et al. [24].

3. Results

The results of initial seroprevalence of brucellosis in goat flocks at Xaltepec are shown in **Tables 1** and **2**. The seroprevalence in the three groups determined by the 3% RBPT as presumptive test resulted in 22.1, 26.1, and 16.0% (95%CI: 16.5–28.9, 19.9–33.2, and 11.1–22.3, respectively).

The serum positive goats were confirmed with SRD, and the prevalence reduced to 0.5, 1.1, and 2.2% (95%CI: 0.3–3.4, 0.1–4.3, and 0.7–5.9, respectively). Thus, a general prevalence of 1.2% (95%CI: 0.5–2.7) was observed.

Tables 3–6 show the seroconversion rate in goats vaccinated with *Brucella* strain determined by RBPT at 30, 60, 90, and 365 days after vaccination. At 30 days after vaccination, the 77.7% (95%CI: 61.9–88.2) of goats vaccinated with Rev. 1 strain became positive to RBPT. Thereafter, 60 and 90 days post vaccination the percentage of seropositive goats declined to 72.2% (95%CI: 56.0–84.1) and 63.8% (95%CI: 47.5–77.5), respectively. At 365 days, 13.8% of vaccinated animals remained as seropositive to RBPT. Only two animals vaccinated with RB51 and RB5-SOD, respectively, were positive to RBPT at 30, 60, and 90 days after vaccination with

Strain	Sample size	RBPT		
		Positive	Seroprevalence (%)	95%CI
Rev. 1	185	41	22.1	16.5–28.9
RB51	180	47	26.1	19.9–33.2
RB51-SOD	181	29	16.0	11.1–22.3
Total	546	117	21.4	18.1–25.1

Table 1.
 Seroprevalence of brucellosis rate by RBPT in goat flocks of Xaltepec municipality Perote, Veracruz, Mexico.

Strain	Sample size	SRD		
		Positive	Seroprevalence (%)	95%CI
Rev. 1	41	1	0.5	0.3–3.4
RB51	47	2	1.1	0.1–4.3
RB51-SOD	29	4	2.2	0.7–5.9
Total	117	7	1.2	0.5–2.7

Table 2.
 Seroprevalence of brucellosis rate by SRD in goat flocks of Xaltepec municipality Perote, Veracruz, Mexico.

Group/subgroup	N	Time after vaccination (days)			
		30			
		Positive	Seroconversion rate (%)	95%CI	
Rev 1	Vaccinated	36	28 a	77.7	61.9–88.2
	Control	36	4 b	11.1	4.4–25.3
RB51	Vaccinated	36	1 a	2.7	0.4–14.1
	Control	36	0 a	0.0	0.0–0.09
RB51-SOD	Vaccinated	36	2 a	5.5	1.5–18.1
	Control	36	0 a	0.0	0.0–0.09

Different superscripts indicate statistical difference by column (P < 0.01).

Table 3.
 Seroconversion rates determined by RBPT at 30 days after vaccination of goats with Rev-1, RB51, and RB51-SOD strains in Xaltepec, Perote, Veracruz, Mexico.

a prevalence of 2.7% and 5.5% (95%CI: 0.4–14.1 and 1.5–18.1, respectively). At 365 days post vaccination, only 11.1% of vaccinated animals with RB51 remained reacting; there were no seroreactors to RB51-SOD strain by RBPT. Meanwhile, animals vaccinated with RB51 and RB51-SOD did not produce anti-O side-chain antibodies as measured by RBPT. Non-vaccinated control goats were seronegative. The seroconversion of a vaccine is the persistent serological reaction, especially when animals are vaccinated as adults; these persistent serological reactions are mainly against the antigenic O-chain of the lipopolysaccharide present in smooth *Brucella* strains [20].

Tables 7–10 show positive animals to RBPT that were confirmed with the SRD at 30, 60, 90, and 365 days after vaccination. Only 2.7% (95%CI: 0.4–14.1) of goats

Group/subgroup		N	Time after vaccination (days)		
			60		
			Positive	Seroconversion rate (%)	95%CI
Rev 1	Vaccinated	36	27 a	72.2	56.0–84.1
	Control	36	4 b	11.1	4.4–25.3
RB51	Vaccinated	36	1 a	2.7	0.4–14.1
	Control	36	0 a	0.0	0.0–0.09
RB51-SOD	Vaccinated	36	2 a	5.5	1.5–18.1
	Control	36	0 a	0.0	0.0–0.09

Different superscripts indicate statistical difference by column (P < 0.01).

Table 4. Seroconversion rates determined by RBPT at 60 days after vaccination of goats with Rev-1, RB51, and RB51-SOD strains in Xaltepec, Perote, Veracruz, Mexico.

Group/subgroup		N	Time after vaccination (days)		
			90		
			Positive	Seroconversion rate (%)	95%CI
Rev 1	Vaccinated	36	23 a	63.8	47.5–77.5
	Control	36	4 b	11.1	4.4–25.3
RB51	Vaccinated	36	1 a	2.7	0.4–14.1
	Control	36	0 a	0.0	0.0–0.09
RB51-SOD	Vaccinated	36	2 a	5.5	1.5–18.1
	Control	36	0 a	0.0	0.0–0.09

Different superscripts indicate statistical difference by column (P < 0.01).

Table 5. Seroconversion rates determined by RBPT at 90 days after vaccination of goats with Rev-1, RB51, and RB51-SOD strains in Xaltepec, Perote, Veracruz, Mexico.

Group/subgroup		N	Time after vaccination (days)		
			365		
			Positive	Seroconversion rate (%)	95%CI
Rev 1	Vaccinated	36	5 a	13.8	6.0–28.6
	Control	36	9 a	25.0	13.7–41.0
RB51	Vaccinated	36	4 a	11.1	4.4–25.3
	Control	36	9 a	25.0	13.7–41.0
RB51-SOD	Vaccinated	36	0 a	0.0	0.0–0.09
	Control	36	7 a	19.4	9.7–35.0

Different superscripts indicate statistical difference by column (P < 0.01).

Table 6. Seroconversion rates determined by RBPT at 365 days after vaccination of goats with Rev-1, RB51, and RB51-SOD strains in Xaltepec, Perote, Veracruz, Mexico.

vaccinated with Rev. 1 became positive during the first three samplings, but this situation did not persist until at 365 days post vaccination as expected. Also, goats vaccinated with RB51 and RB51-SOD during the first 90 days post vaccination expressed

Group/subgroup		Time after vaccination (days)		
		30		
		Positive	Prevalence rate (%)	95%CI
Rev 1	Vaccinated	1/28	2.7	0.49–14.1
	Control	0/4	0.0	0.0–0.49
RB51	Vaccinated	0/1	0.0	0.0–0.79
	Control	0/0	w.d.*	w.d.*
RB51-SOD	Vaccinated	0/2	0.0	0.0–0.66
	Control	0/0	w.d.*	w.d.*

*w.d. = without data

Table 7.
 Seroconversion rates determined by SRD at 30 days after vaccination of goats with Rev-1, RB51, and RB51-SOD strains in Xaltepec, Perote, Veracruz, Mexico.

Group/subgroup		Time after vaccination (days)		
		60		
		Positive	Prevalence rate (%)	95%CI
Rev 1	Vaccinated	1/27	2.7	0.49–14.1
	Control	0/4	0.0	0.0–0.49
RB51	Vaccinated	0/1	0.0	0.0–0.79
	Control	0/0	w.d.*	w.d.*
RB51-SOD	Vaccinated	0/2	0.0	0.0–0.66
	Control	0/0	w.d.*	w.d.*

*w.d. = without data

Table 8.
 Seroconversion rates determined by SRD at 60 days after vaccination of goats with Rev-1, RB51, and RB51-SOD strains in Xaltepec, Perote, Veracruz, Mexico.

Group/subgroup		Time after vaccination (days)		
		90		
		Positive	Prevalence rate (%)	95%CI
Rev 1	Vaccinated	1/23	2.7	0.49–14.1
	Control	0/4	0.0	0.0–0.49
RB51	Vaccinated	0/1	0.0	0.0–0.79
	Control	0/0	w.d.*	w.d.*
RB51-SOD	Vaccinated	0/2	0.0	0.0–0.66
	Control	0/0	w.d.*	w.d.*

*w.d. = without data

Table 9.
 Seroconversion rates determined by SRD at 90 days after vaccination of goats with Rev-1, RB51, and RB51-SOD strains in Xaltepec, Perote, Veracruz, Mexico.

antibodies that were detected with the RBPT test but were negative to the SRD test; however, at 365 days, an animal in the RB51 strain group was identified as seropositive (2.7%, 95%CI: 0.4–14.1). It is noteworthy that serological samples that underwent

Group/subgroup		Time after vaccination (days)		
		365		
		Positive	Prevalence rate (%)	95%CI
Rev 1	Vaccinated	0/5	0.0	0.0–0.43
	Control	0/9	0.0	0.0–0.29
RB51	Vaccinated	1/4	2.7	0.49–14.1
	Control	1/9	2.7	0.49–14.1
RB51-SOD	Vaccinated	0/0	w.d.*	w.d.*
	Control	1/7	2.7	0.49–14.1

*w.d. = without data

Table 10.

Seroconversion rates determined by SRD at 365 days after vaccination of goats with Rev-1, RB51, and RB51-SOD strains in Xaltepec, Perote, Veracruz, Mexico.

the confirmatory test (SRD) correspond to animals that had a positive result to the screening test (RBPT); hence, the original sample size was not decreased.

4. Discussion

Goat herds in the present study had similar conditions of feeding, handling, and confinement. Each group was exposed to animals infected with *Brucella* spp. Overall seroprevalence in the herds under study was 21.4% (95%CI: 18.1–25.1) with 3% RBST as screening and 1.2% (95%CI: 0.5–2.7) by SRD as the confirmatory one. These seroprevalences are similar to those found by Román-Ramírez et al. in 14 municipalities in the central area of the state of Veracruz, Mexico, that were 18.18% (95%CI: 15.15–21.64) by RBST and 0.52% (95%CI: 0.13–1.65) by SDR tests [12]. However, the seroprevalence is also greater than 9.8% reported by Solorio-Rivera et al. (95%CI: 8.8–10.7) [5] using RBST test in goat herds of the state of Michoacán, Mexico. This shows that the herds located in the community of Xaltepec, municipality of Perote, Veracruz, Mexico, have animals that could be exposed to brucellosis and the conditions of management provide an opportunity for the perpetuating the infection.

The permanent vaccination program for goat herds has been operating in the area since 1994 achieving the requirements for the control phase according to the Official Mexican Standard (NOM-041-ZOO-1995) National Campaign against brucellosis in animals. These findings may suggest that the vaccine used is not protecting all animals, the vaccine is not properly managed or injected, or vaccination is not timely applied, resulting in the possibility of maintaining infection in the animals. Furthermore, the animal may not develop the infection, but the immune response capability is then detected by the diagnostic screening test without being a truly infected animal. As a result, the recognized agglutination serological tests (RSBT) leads to diagnostic confusion determining infected animals to remain in the herds. Hence, it is necessary to evaluate the vaccine strain to be used in the brucellosis control programs, since the results shown in **Table 1** demonstrate that more than 50% of the animals reacted to the screening test, but are not infected as shown by the SRD test (**Tables 7–9**), which possess a greater sensitivity. This situation determines the need to invest in confirmatory tests [25–29].

When vaccinated groups of goats were evaluated by the RSBT, animals vaccinated with Rev. 1 strain had a seroconversion rate of 77.7% (95%CI: 61.9–88.2),

72.2% (95%CI: 56.0–84.1), 63.8% (95%CI: 47.5–77.5), and 13.8% (95%CI: 6.0–28.6) at 30, 60, 90, and 365 days post vaccination, respectively (**Tables 3–6**). This agrees with Blasco et al. [7] who pointed out that vaccination with a full dose (1×10^9 CFU/mL) may cause diagnostic interference and inconvenience to rely on vaccination as the only alternative for brucellosis eradication programs in goat herds [7, 27]. RBST-positive animals were confirmed by the SRD test, and only one animal resulted positive, representing 2.7% (95%CI: 0.4–14.1) (**Tables 7–9**). This indicates that the vaccine did not protect or that the animal was infected prior to vaccination, despite being negative at initial screening. Vaccinated animals were not challenged at a controlled dose of *Brucella melitensis*, since the challenge was through a natural exposure to the infected animals, which were kept in confinement with the vaccinated animals, to allow exposed vaccinated animals to become infected as occurring in the normal management situation in the regional production systems in Mexico [14, 30].

As observed in **Tables 3–5**, animals vaccinated with the RB51 and RB51-SOD strains, 2.7% (95%CI: 0.4–14.1) and 5.5% (95%CI: 1.5–18.1), respectively, reacted to the RBST during the evaluation period. However, when analyzed by the SRD for confirmation, all animals were negative. RB51 strain is officially used for vaccination only in bovine females; it is a rough mutant strain derived from *B. abortus* 2308 smooth strain, so it does not induce response of anti-LPS antibodies. It has the advantage of allowing conventional serological tests to be used for brucellosis diagnosis in animals, and its use is considered safe in small ruminants [31]. Fosgate et al. carried out a study in water buffalo males and females which were vaccinated subcutaneously with RB51 at a concentration of $1.0\text{--}3.4 \times 10^{10}$ UFC/mL, to evaluate the serological performance by agglutination tests [31]. Animals were challenged in a herd with an initial *Brucella* spp. prevalence of 56%. Out of the vaccinated animals, 2/32 (6.2%) reacted in different samplings in at least one serological test (STAT, SPAT, and/or BPAT). Authors conclude that the proportion of vaccinated animals that became positive to brucellosis in this field trial was greater than the corresponding proportion in the control group emphasizing that vaccination does not stop the seroconversion effect on the herds challenged with a field strain. Furthermore, the RB51 vaccine did not prevent seroconversion of the animals. Therefore, infected animals were able to process the agent and maintain such a condition that it could react to the diagnostic test by IgM production by stimulation of the O-type side chains of the field strain, although the animal was not infected [28, 29, 32].

El Idrissi et al. compared the vaccine efficacy of Rev. 1 and RB51 strains in sheep. Considering seroconversion, they conclude that after vaccination, all sheep vaccinated with Rev. 1 were positive to RBPT and complement fixation test (CFT) at 2 weeks, reaching their maximum between 2 and 6 weeks [7]. Then the percentage decreased and was zero 14 weeks after challenge. Animals vaccinated with RB51 did not produce anti-O side-chain antibodies, as measured by RBPT and CFT. After exposure to challenge, anti-O side-chain antibodies, measured by RBPT, were detected in the serum of vaccinated animals and controls [19].

Out of the animals vaccinated with RB51-SOD strain, 2/36 were seroconverted, representing 5.5% (95%CI: 1.5–18.1) (**Tables 3–5**). The animals that underwent the confirmatory test (SRD) were negative as shown in **Tables 7–9**. The above indicates that animals established an immune memory response generating the production of immunoglobulins detectable by the screening test, but they were not infected [34]. Olsen et al. [32] evaluated the RB51-SOD strain in bisons, which was less effective than RB51 in protecting against abortion and uterine infection in this species [32–34]. In the present study, some animals of the goat groups of *B. abortus* strains RB51 and RB51-SOD were positive only to

the screening test, which could be discarded by SRD test that identified them as negative to brucellosis [28, 29, 31, 33, 34].

The RB51-SOD strain was obtained from *B. abortus* 2308 in order to generate the overexpression of a protective periplasmic antigen of the protective antigen known as Cu/Zn SOD, which causes the immune cell response by T-helper-type Th1 lymphocytes, and protection against the strain of *B. abortus* 2308, which has been demonstrated in murine models [26, 29, 31–33]. Despite the favorable outcome in mice, Dorneles et al. [33] pointed out that the potential use of RB51-SOD under field conditions is very limited, although satisfactory results have been obtained. It is important to consider that the response observed in the mice might not reflect the protection achieved in the natural hosts after vaccination. Moreover, to generate a strong and protective immune response that mimic natural infection is a complex challenge. However, the current study in goats allowed to evaluate the RB51-SOD strain and to know part of its satisfactory performance in the field, since the newly developed vaccines have only evaluated in murine models [28–30]. Contrary to the Rev. 1 vaccine, current study demonstrates that the RB51-SOD strain does not induce seroconversion in goats.

5. Conclusion

When evaluating the Rev. 1, RB51, and RB51-SOD vaccine strains, seroconversion in animals vaccinated with Rev. 1 strain was higher than that shown by the strains RB51 and RB51-SOD by conventional serological tests in infected herds during the evaluated period. Therefore, vaccination with Rev. 1 originates the need to perform confirmatory tests causing an increase in diagnosis costs. According to results of the present study, the RB51-SOD vaccine represents an alternative for controlling one of the most important worldwide zoonosis in goats. However, further studies are required to evaluate the performance of immune response, vaccine safety, and efficacy at field level.

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Conflict of interest

The authors have no conflict of interest to declare.

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