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Article

Evaluation of productive indicators in goat herds vaccinated with RB51–SOD, RB51 (*Brucella abortus*) and Rev-1 (*Brucella melitensis*) strains

Baldomero Molina-Sánchez [a]

David Izcoatl Martínez-Herrera [a]*

Violeta Trinidad Pardío-Sedas [a]

Ricardo Flores-Castro [b]

José A. Villagómez-Cortés [a]

José F. Morales-Álvarez [c]

[a] Universidad Veracruzana. Facultad de Medicina Veterinaria y Zootecnia. Av. Miguel Ángel de Quevedo s/n, esq. Yáñez, Col. Unidad Veracruzana, 91710, Veracruz, Veracruz, México.

[b] Laboratorios Tornel, S.A. de C.V., Naucalpan de Juárez, Estado de México, México.

[c] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad (CENID-SAI), Ciudad de México, México.

*Corresponding author: dmartinez@uv.mx

Abstract:

Kidding rates, miscarriages and births of weak offspring were determined in herds vaccinated with the RB51-SOD (*B. abortus*) strain in order to evaluate the productive improvement and compare it with Rev-1 (*B. melitensis*) and RB51 (*B. abortus*) vaccines. Three subgroups of 36 goats each were vaccinated with Rev-1 (1-2x109 CFU), RB51 (3x108-3x109 CFU) and RB51-SOD (3x108-3x109 CFU) strains, with each strain having a control subgroup. Individual records were established for calculating post-vaccination rates in two kidding seasons. In the first, the kidding rate for Rev-1 was 66.6 % (95%CI: 48.9-80.9), RB51

50.0 % (95%CI:33.2-66.7), and RB51-SOD 69.4 % (95%CI:51.7-83.0). Miscarriages occurred in all three vaccinated subgroups, Rev-1 and RB51-SOD 5.5 % (95%CI: 0.9-20.0) and RB51 2.7 % (95%CI: 0.1-16.2). Weak offspring births occurred only in animals vaccinated with Rev-1 5.5 % (95%CI: 0.9-20.0). During the second epoch, the kidding rate in Rev-1 vaccinated females was 91.6 % (95% CI:76.4-97.8), RB51 94.4 % (95% CI:79.9-99.0), and RB51-SOD 94.4 % (95% CI:79.9-99.0). Animals vaccinated with Rev-1 and RB51 strains had 5.5 % (95%CI: 0.9-20.0) and 2.7 % (95%CI: 0.1-16.2) miscarriages, respectively; in vaccinated subgroups there were no births of weak offspring. The control subgroups behaved similarly to the vaccinated subgroups. Animals vaccinated with the RB51-SOD strain showed no significant difference from those that received the Rev-1 and RB51 strains, nor from the control subgroups (P>0.01); therefore, the RB51-SOD vaccine can generate protection against brucellosis and benefits in the production of goat herds.

Key words: Vaccination, Abortions, Brucellosis, Goats, RB51- SOD.

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Introduction

Brucellosis is an emerging and globally distributed disease that is considered among the 10 zoonoses neglected by health authorities[1,2]. From an economic point of view, it is important because of the effects it has on animal production units, as well as the risk it poses for the human population.[3]. Goat producers state that the activity presents technological and sanitary lags, and highlight the persistence of goat brucellosis, which reduces productivity, lowers milk quality and represents a risk of infection for humans[4]. Bacteria of the genus *Brucella* cause the disease; the most virulent species are *Brucella melitensis* and *Brucella abortus*, responsible for the disease in small ruminants and cattle, respectively[5]. The clinical manifestation of infection in pregnant animals includes miscarriage, birth of offspring that die in peripartum, and arthritis[6,7]. In affected goat herds, low productive efficiency is observed due to the infertility caused in infected animals; miscarriages increase by up to 20%, and the productive capacity of sick females decreases by up to 30%[8,9].

The low kidding rate is the result of miscarriages that occur due to sanitary conditions, including the persistent prevalence of brucellosis and severe nutritional restriction during gestation[10-13]. In herds infected with brucellosis, vaccination, diagnosis and selective slaughter of animals are alternatives for the control or eradication of the disease[2]. Currently,

the Rev-1 strain of *Brucella melitensis* is a modified live strain used to control the infection in sheep and goats. However, it has limitations, such as the ability to induce abortionin pregnant females, be excreted into the milk, infect humans, and potentially be resistant to streptomycin, an antibiotic that, in combination with doxycycline, is the most effective treatment for brucellosis in humans[12,14,15].

Brucella abortus strain RB51 is used for the control of brucellosis in cattle and has been evaluated in small ruminants under controlled conditions with good protection against experimental challenge with *B. melitensis*[14]. There is information that sustains that the protection conferred is lower than that obtained with the Rev-1 strain and that it causes miscarriages and stillbirths in goats[14]. However, it has the advantage of not producing post-vaccination diagnostic interference, compared to conventional serology[16,17,18].

DNA plasmid vaccines have the potential to be the future for brucellosis control. Homologous overexpression strains have been evaluated to induce an immune response. Overexpression of Cu/Zn SOD (superoxide dismutase), which is a periplasmic protein that has developed protection, in murine models, against experimental infection with virulent *B. abortus* strain 2308 has been shown to achieve protection equal to that induced by RB51 (*B. abortus*)[16,19]. Oñate *et a.*[19] evaluated the SOD strain in cattle and obtained antibody response and Th-1 type MIC, and protection against *B. abortus* challenge. Further studies are required to know the role of different types of T cells in the protection induced by vaccination with pcDNA-SOD and its results in productive systems[19-22]. Immune response and vaccine efficacy may differ between laboratory animals and susceptible ruminants[22]. Because there is a lack of information on the use of the RB51 - SOD strain in domestic animals, its effects and benefits, as well as its safety in preventing abortion induction by effect of the vaccine and protection for the improvement of production in goat herds. The objective of this study was to determine farrowing rates, miscarriages and births of weak offspring in herds vaccinated with the RB51-SOD strain (*Brucella abortus*) in order to evaluate the productive improvement and compare it with that obtained when using the Rev-1 (*Brucella melitensis*) and RB51 (*Brucella abortus*) vaccines.

Material and methods

Study area

The study was conducted in goat production units in the community of Xaltepec in the municipality of Perote, located in the central zone of the state of Veracruz, Mexico. The community is located at the coordinates 97°21'22.21.21" W and 19°22'50.06.06" N, and at

an altitude of 2,358 masl, and it borders the state of Puebla; its climate is cold and dry, with an average annual temperature of 12 ºC and an average annual rainfall of 493.6 mm[23].

The main livestock activity is goat and sheep production, under a semi-stabled system where the owners and family members tend to the animals. The herds are composed of an average of 64 goats, most of which graze communal land. During the peak fodder-production season, some producers confine them in order to use the agricultural residues produced in the area. The main production is milk for cheese production, and meat through the sale of kids at weaning and of cull females[24].

Study type and sample size

The study was a Phase III clinical trial conducted from September 2016 to March 2018 to evaluate farrowing rates, miscarriages, and weak offspring births in brucellosis-positive goat herds vaccinated with *Brucella abortus* RB51-SOD, *Brucella abortus* RB51, and *Brucella melitensis* Rev-1 strains. The sample size was estimated using the Win Episcope Ver. 2.0 program, a prevalence of 0.52 % in goats having been found in a previous study in that area of Veracruz[24], with a 95% confidence interval and an error of 5%. The minimum sample size was 72 goats for each treatment group (strain); each block consisted of a vaccinated subgroup (36) and a control subgroup (36). Each group studied consisted of goats aged over three months that tested seronegative for brucellosis and had never been vaccinated. The brucellosis-seropositive animals identified during an initial sampling performed prior to vaccination to determine seropositive animals were kept in the herds in order to undergo permanent exposure along with the susceptible animals. The animals in each group were identified with metal earrings in the left ear.

The research protocol was reviewed and approved by the Bioethics Commission of the Faculty of Veterinary Medicine and Animal Husbandry of Universidad Veracruzana.

Vaccination

Animals in the vaccinated subgroups of each group were administered 2 ml of vaccine subcutaneously on the left side of the middle third of the neck. The first group received the Rev-1 strain of *Brucella melitensis* at doses of $1 – 2x10^9$ CFU; the second group received the RB51 strain of *Brucella abortus* at doses of $3x10^8$ to $3x10^9$ CFU; and the third group received the RB51-SOD strain of *Brucella abortus* at doses of $3x10^8$ to $3x10^9$ CFU. The latter vaccine was imported for research purposes by the National Center for Disciplinary Research in Animal Health and Safety (Centro Nacional de Investigación Disciplinaria en Salud Animal

e Inocuidad, CENID-SAI) of the National Institute for Research on Forestry, Agriculture and Livestock (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, INIFAP) and was provided by Dr. Gerhardt Shurig, of the Virginia Polytechnic Institute and State University. Each vaccinated subgroup had its control subgroup, *i.e.* animals that received 2 ml of physiological saline solution subcutaneously in the left side of the middle third of the neck as a placebo.

Tracking of individual records

Individual records were kept for each of the animals in the sample, in accordance with INIFAP's recommendations for the reproductive management of goats on pasture[25], in order to record the dates of births, miscarriages and issues during gestation. Gestation, parturition and abortion rates were calculated to determine the initial situation of the herds, considered as baseline information for the study. Likewise, the animals in the sample were monitored daily for two parturition periods, in order to determine post-vaccination behavior, as well as for indicators such as parturition, miscarriages and births of weak offspring; the dates corresponded to the seasonal kidding periods defined in the herds (October - February)

Calculation of kidding rates, miscarriages and births of weak offspring

Kidding rates, miscarriages and births of weak offspring in the three groups were integrated by using the information included in the individual records. Differences between groups and the significance of association were estimated based on categorical data analysis (Chi^2) and the degree of association by Relative Risk (RR)[26].

Results

Table 1 shows the productive indicators identified in the goat herds in the community of Xaltepec, in the municipality of Perote, prior to vaccination of the animals with the experimental strains, comprising 529 head of goats. The average gestation, kidding and abortion rates were 69.2, 95.2 and 2.7 %, respectively; this information was considered as a baseline for the herds under study. The herds utilized had an overall prevalence of brucellosis confirmed by the radial immunodiffusion test (RID) of 1.2% (95%CI: 0.5- 2.7)[27].

Table 1: Inventory and reproductive indicators in herds in the community of Xaltepec, Perote, Veracruz, Mexico, prior to vaccination

Strain	Inventory (animals)	Rate		
		Pregnancy	Births	Abortions
Rev – 1	134	67.5	96.5	2.0
RB51	192	69.0	95.6	3.0
RB51– SOD	203	71.3	93.6	3.3
Total	529			
Average		69.2	95.2	2.7

After vaccination, production indicators were evaluated in the herds during two kidding seasons. Table 2 shows the rates for kidding, abortions and births of weak offspring during the first kidding period, and it may be observed that the vaccinated subgroups had a similar behavior in relation to kidding and abortion rates. However, in the animals vaccinated with the Rev-1 strain, there was a rate of 5.5 % percentage (95%CI: 0.9 - 20.0) of weak offspring births, but in the subgroups vaccinated with the RB51 and RB51-SOD strains this condition did not occur. The control subgroups showed similar behavior to the vaccinated subgroups.

Table 3 shows the relative risk (RR) and Chi^2 for births, abortions and weak-kids born in the first post-vaccination kidding period and shows that there was no significant difference (P>0.01) between vaccinated and control subgroups.

Table 2: Production indicators during the first kidding period in goat flocks vaccinated with different strains in the community of Xaltepec, Perote, Veracruz, Mexico

Strains	Group	N	Births			Abortions			Weak offspring		
			No.	%	(95% CI)	No.	%	(95% CI)	No.	%	(95% CI)
Rev 1	Vaccinated	36	24	66.6	48.9 – 80.9	2	5.5	0.9 – 20.0	2	5.5	0.9 – 20.0
	Control	36	22	61.1	43.5 – 76.3	1	2.7	0.1 – 16.2	0	0.0	0.0
RB51	Vaccinated	36	18	50.0	33.2 – 66.7	1	2.7	0.1 – 16.2	0	0.0	0.0
	Control	36	21	58.3	40.8 – 74.0	1	2.7	0.1 – 16.2	0	0.0	0.0
RB51 – SOD	Vaccinated	36	25	69.4	51.7 – 83.0	2	5.5	0.9 – 20.0	0	0.0	0.0
	Control	36	24	66.6	48.9 – 80.9	1	2.7	0.1 – 16.2	0	0.0	0.0

Table 3: Relative Risk and Chi^2 values of births, abortions and births of weak offspring from the first post-vaccination kidding period

Strain	Births			Abortions			Weak offspring		
	RR	($IC_{95\%}$)	Chi^2	RR	($IC_{95\%}$)	Chi^2	RR	($IC_{95\%}$)	Chi^2
Rev 1	0.4	0.1 – 1.3	2.68	2.0	0.2 – 21.1	0.4	0.9	0.1 – 2.9	0.4
RB51	0.4	0.1 – 2.0	1.42	1.0	0.1 – 15.4	0.0	0.0	0.0	0.0
RB51–SOD	0.5	0.1 – 2.6	0.73	2.0	0.2 – 21.1	0.4	0.0	0.0	0.0

(P<0.01).

In the second kidding period, the performance of the productive indicators of the vaccinated females and the control subgroups was evaluated as shown in Table 4, where it is observed that the vaccinated subgroups improved the indicator in relation to the kidding rate compared to the first kidding period. Miscarriages occurred only in animals vaccinated with the Rev-1 strain and in their control group, 2.7 % (95%CI: 0.1-16.2) and 5.5 % (95%CI: 0.9-20.0), respectively. Animals vaccinated with the RB51 and RB51-SOD strains had no abortions; however, the RB51 strain control group had a rate of 2.7 % (95%CI: 0.1-16.2). In the three vaccinated subgroups, as well as in the controls, there were no cases of birth of weak offspring. In Table 5, the statistical analysis of these results reveals that there is no significant difference between vaccinated subgroups and controls (P>0.01).

Table 4: Production indicators during the second kidding period in goat flocks vaccinated with different strains

Strain	Group	N	Births			Abortions			Weak offspring		
			N	%	($_{95\%}$ CI)	N	%	($_{95\%}$ CI)	N	%	($_{95\%}$CI)
Rev 1	Vaccinated	36	33	91.6	76.4 – 97.8	1	2.7	0.1 – 16.2	0	0.0	0.0
	Control	36	28	77.7	60.4 – 89.2	2	5.5	0.9 – 20.0	0	0.0	0.0
RB51	Vaccinated	36	34	94.4	79.9 – 99.0	0	0.0	0.0	0	0.0	0.0
	Control	36	31	86.1	69.7 – 94.7	1	2.7	0.1 – 16.2	0	0.0	0.0
RB51 – SOD	Vaccinated	36	34	94.4	79.9 – 99.0	0	0.0	0.0	0	0.0	0.0
	Control	36	32	88.8	73.0 – 96.3	0	0.0	0.0	0	0.0	0.0

Table 5: Relative Risk (RR) and Chi^2 values for parturitions, abortions and weak-born kids of the second post-vaccination parturition period

Strain	Births			Abortions			Weak offspring		
	RR	(95% CI)	Chi^2	RR	(95% CI)	Chi^2	RR	(95% CI)	Chi^2
Rev 1	0.3	0.1 – 1.3	2.7	0.5	0.1 – 5.3	0.4	0.0	0.0	0.0
RB51	0.4	0.1 – 1.9	1.4	0.0	0.0	0.0	0.0	0.0	0.0
RB51–SOD	0.5	0.1 – 2.6	0.7	0.0	0.0	0.4	0.0	0.0	0.0

(P>0.01).

Discussion

In the flocks of the experiment, the animals that tested seropositive to the confirmatory serological test of radial immunodiffusion (RID) for brucellosis diagnosis, remained in their herds of origin during the whole period of the study, in order to allow the natural, direct challenge of vaccinated animals and controls in the flocks that had an overall seroprevalence of 1.2 % (95%CI: 0.5 - 2.7)[27]. This value is higher than the general average of 0.52 % (95%CI: 0.1 - 1.6) found in 14 municipalities in the central zone of the state of Veracruz and the 0.05 % reported by SENASICA at the national level in goat flocks[24]. Exposure of vaccinated females within infected herds allows challenging the protection conferred under natural conditions; in this study, the challenge to the field strain by the experimental animals was assessed through seroprevalence confirmed with the SRD test[27]. For the challenge of experimental herds in field conditions, it is necessary to consider the seroprevalence of the disease detected with more specific (confirmatory) tests; otherwise, the challenge of vaccinated herds with seropositive animals is not guaranteed. This is because when using only a screening test, there is the possibility of having false positive animals considered as infected. In this case, the seropositivity may be due to the window generated by the seroconversion as a consequence of vaccination with strains that have this characteristic in those animals, or even of cross-reactions with other microorganisms, and therefore may hinder correct discrimination between infected and merely reactive animals[16,17].

Table 1 shows the farrowing and abortion rates, which average 96.6 % and 1.8 %, respectively. This behavior is similar to that reported by other researchers in goat farming in the states of Oaxaca and Nuevo León, located in the two regions that account for 70.2 % of the national goat inventory[4,28]. However, herds characterized as under pasture conditions in the country have a gestation rate of less than 65 %, as a result of abortions, poor sanitary conditions and severe nutritional restriction during pregnancy[11]. Comparison between the results of the vaccinated subgroups and controls with the indicators observed in the present

study in the Xaltepec goat farm shows that it is not sufficient to establish vaccination programs to prevent or control diseases such as brucellosis: adequate feeding according to the breed characteristics of the animals that make up the herd and the production system must be also included in order to improve their productivity. In addition, the improvement in the indicators of kidding, abortions or births of healthy offspring in the first post-vaccination kidding period cannot be attributed exclusively to vaccination[11,13].

During the first post-vaccination kidding period, the kidding rate in all groups that were part of the clinical trial, both vaccinated and controls, was lower compared to the initial indicators, because not all females in the experiment entered mating, possibly due to their age, poor body condition and nutritional status —a situation that coincides with the effects of malnutrition during development and early postnatal life-, since this causes permanent and irreversible effects during puberty, as well as in the adult life of smaller ruminants[13]. Abortions occurred in both subgroups of animals vaccinated with Rev-1 (*B. melitensis*) and RB51-SOD (*B. abortus*) strains. In the control subgroups, abortions also occurred, at a rate of 2.7 % (95%CI: 0.1 - 16.2). When comparing between the vaccinated and control groups, no significant difference was found ($P<0.01$).

Birth of weak offspring occurred only in two females that were vaccinated with the Rev-1 strain; this is a condition that can occur in the offspring of brucellosis-infected animals —a situation that coincides with the results of serology performed by the SDR test, where two females had positive serology[6,27]. Fetal losses and miscarriages in goat herds are the main reproductive issue, which is caused not only by infectious agents but also by nutritional stress in goats[11]. In addition, malnutrition affects animals exposed to vaccine or field strains by preventing the animal from producing antibodies that can be measured by conventional serological tests, or from establishing protection against the causal agent[29-33].

During the second kidding period after vaccination, the kidding rates observed in the three groups exhibited similar behavior. However, abortions occurred in the Rev-1 vaccine and control subgroups, 5.5 % (95%CI: 0.9 - 20.0) and in the RB51 control subgroup, 2.7 % (95%CI: 0.1 - 16.2). No vaccinated subgroup gave birth to weak offspring. Statistical analysis showed that there was no significant difference ($P<0.01$). It should be noted that vaccination status is not associated with the presence of abortion. Villa *et al*[17] conducted the evaluation of vaccines for the control of brucellosis and found that the RB51 strain (*B. abortus*) had a abortion rate of 74.2 %, which is considered high, in addition to presenting the highest relative risk of abortion, compared to the results of other studies in which goats and pregnant sheep were vaccinated with this strain and abortion rates of less than 10 % were found[29,31,33]. Therefore, this vaccine is not recommended for use in goats. However, this information differs from the findings of this study in the community of Xaltepec, since, according to other studies, its application in small ruminants can cause up to 1% of abortions in susceptible females due to vaccine effect[31]. Thus, it is advisable to vaccinate females older than three

months and not pregnant, a condition that must be met in order to avoid the risk of abortion due to vaccine effect —a situation that some countries have established as a rule in order to avoid economic losses[18]. The RB51 vaccine strain (*B. abortus*) is authorized for use in Mexico only for the bovine species. However, in 2005, the health authority registered a vaccine with these characteristics for use in goats[34] and it agrees with this study in that the strain is safe because it did not produce abortion in the females of the corresponding subgroup in the community of Xaltepec.

There is information indicating that up to 2 % of abortions may occur in susceptible females due to the effect of vaccination[14,31]. However, it is important to consider that there are factors specific to each susceptible individual, such as age, sex, reproductive status, immune and nutritional condition, as well as the agent, which alter the development of the protection generated by the vaccine and, in general, the protection conferred by vaccines against brucellosis, which ranges between 85 and 90 %[30,32,35]. The birth of weak offspring that die during the peripartum is a condition that can occur in the offspring of brucellosis-infected animals; this agrees with the results of a serology performed using the SDR test, where two females tested positive[6,27].

The Rev-1 (*B. melitensis*) and RB51 (*B. abortus*) strains have been evaluated for their protection and side effects in vaccinated animals. Today, DNA plasmid vaccines have been developed that offer an alternative to homologous overexpression vaccines such as the RB51-SOD (CU/Zn) strain used in this study, which in murine models shows better protection against *B. abortus* than that established by RB51[19] and is considered one of the DNA vaccines that demonstrate the capacity to produce cellular and humoral immunity and a certain degree of protective immunity[16,31,33]. In cattle, it suggests the production of antibodies and Th-1 type MICs, and generates protection in vaccinated animals when challenged with *B. abortus*[19]. When evaluating the behavior of the RB51 - SOD strain in the field in goat herds with seroprevalences of 1.2 % (95%CI: 0.5 - 2.7), the animals vaccinated with this strain did not present abortion, nor births of weak offspring. It is important to point out that according to published results, animals vaccinated with this strain do not seroconvert to conventional tests at 90 d post-vaccination, and therefore do not generate diagnostic confusion[27].

Regarding the effect on increased kid births, there was no significant difference ($P<0.01$) between goats vaccinated with the Rev-1 (*B. melitensis*) and those administered the RB51 (*B. abortus*) strains. Olsen *et al*[24], when evaluating the RB51-SOD strain in bison and comparing it with the RB51 strain, found no differences in the behavior of both strains, and suggest that the data obtained for the RB51 - SOD strain is safe for this species, since the presence of the vaccine agent in tissues is not observed. However, vaccine efficacy results recommend the RB51 strain as preferable to RB51-SOD for the vaccination of bison

calves[24]. However, its condition for the development of cellular and humoral immunity in goats, as well as its efficacy and safety in young and adult animals need to be evaluated.

Conclusions and implications

When evaluating the rates of kiddings, abortos and births of weak offspring in flocks vaccinated with the RB51 - SOD (*B. abortus*) strain, there was no significant difference between animals inoculated with Rev-1 (*B. melitensis*) and RB51 (*B. abortus*), strains available for use in official Animal Health Campaigns, as well as with the control subgroups. This suggests that the RB51 - SOD strain can generate similar benefits in the protection of the flock against the disease to maintain a healthy inventory and avoid negative effects on the productive life and the health of the females, as well as to guarantee the health of the flock. However, it is necessary to consider complementary activities of management and feeding of the flock to improve productive conditions of the susceptible animals.

Acknowledgments and conflicts of interest

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