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Use of RB51 Vaccine for Small Ruminants Brucellosis Prevention, in Veracruz, Mexico

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Abstract: With the aim to evaluate strain RB51 *Brucella abortus* vaccine under field conditions in goats and ovine communities, located at Perote and Coffer Region in Veracruz, Mexico, where, prevalence rates varies, several vaccination operatives were done between 12 to 15 months, using 3×10^8 to 3×10^9 Colony Forming Units (CFU) of such vaccine for each animal. A total of 5,168 goats or ovine females 3 months old and older, were vaccinated. Those animals belonged to 322 herds in different communities. Vaccination was done despite their reproductive condition. Twenty animals were randomly selected in each herd and blood samples collected, before and after 12 to 15 months of vaccination, in order to determine brucellosis seroprevalence. Sample size for each community was estimated by a probabilistic model, with unknown population and 50% known prevalence rate (n = 1-p/pv); where, n corresponds to sample size, p for prevalence rate and v variation coefficient (0.05), blood samples were taken by jugular vein punction using vacuum tubes system. The sera collected were tested by using 3% antigen concentration card test as screening and complement fixation as confirmation test, according with Mexican regulations. During the first sampling tests it was found that seroprevalence rates in the communities were: 0, 0.5, 4.5, 5, 38 and 1.4%. The rates of seroprevalence in the second sampling were: 0, 0, 5.5, 0 and 0%. The rates of serum reactors were reduced 80% from different rates to 0.0%. According with these results, RB51 *Brucella abortus* strain vaccination provided a successful response at the indicated dosage. It is conclusive that RB51 *Brucella abortus* strain goat and ovine vaccination at communities from Perote and Coffer Region is useful for brucellosis control.

Key words: RB51 vaccine, brucellosis, rate, seroprevalence, reduction

INTRODUCTION

Brucellosis is an infectious disease, very difficult to eradicate and widely distributed around the world. *Brucella abortus* is the first agent for bovine brucellosis with a high

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economic impact for dairy and cattle production, while 90% of Malta Fever or Human Mediterranean Fever cases is due by *Brucella melitensis* with higher impact for public health (Luna and Suárez, 1998); in Mexico 90% of human brucellosis cases are due to *Brucella melitensis* (Bautista *et al*., 2003a), but natural hosts are small ruminants such as goats and sheep. The main agent responsible of caprine and ovine brucellosis is *Brucella melitensis*. A possibility for infection with this microorganism in other domestic and wild mammals is not excluded.

Although, *Brucella melitensis* is a very infectious bacilli it is easily attenuated by heat at 65°C or if it is exposed to 5.0 or less pH environment (Alton *et al*., 1988). Transmission could be direct or indirect by dairy products like fresh chesses which are the most important infection resource, because when they are manufactured, bacteria are trapped into fat milk and plays a role as the major vehicle for *B. melitensis* in human transmission (Gurria, 1998; Lopez, 1998; Meljem and Flores, 1998). Brucellosis is not transmitted among people, thus, human brucellosis is the first signal that *B. melitensis* could be infecting goats and sheep in animal populations (Alton, 1990; Hernández, 1998).

In 1994, Mexican Agriculture Ministry (SAGARPA) improved a national vaccination program. This was to avoid human and animal brucellosis, using *Brucella melitensis* Rev1 vaccine (Castell-Blanch, 1998; Luna and Suárez, 1998; Martinez *et al*., 2001b), during 1994, as a part of the same national vaccination program, the Veracruz State Government coordinated with SAGARPA to promote intensive actions against small ruminants brucellosis, particularly at Perote Coffer and Valley Region, using vaccination as a response to higher human brucellosis cases (Luna and Suárez, 1998; Martinez *et al*., 2000, 2001a).

The evaluation of this program indicated that more than 60% of the Rev1 vaccinated goats were positive to official serological tests after vaccination. It was also demonstrated that some of those vaccinated animal were shedding *Brucella melitensis* biovar 1 through their milk (Martinez *et al*., 2000). For this reason, it was considered that *Brucella melitensis* Rev1 vaccine did not provide to be, under the circumstances studied in Veracruz, a significant tool for reduction of the number of human brucellosis cases (Martinez *et al*., 2001b, 2002).

In view of the results of low protection conferred by Rev1 goat vaccination, another study was carried out to evaluate protection obtained using another kind of vaccines like *Brucella abortus* strain RB51 (Martinez *et al*., 2003a). The RB51 *Brucella abortus* strain was evaluated for efficacy, safety, innocuously, efficiency, protection conferred duration and possibility to revaccinate small ruminants, previously vaccinated with Rev1 vaccine (Martinez *et al*., 2003a, b, 2004a, b).

After an evaluation (Martinez *et al*., 2003a), it was demonstrated the efficiency of RB51 *Brucella abortus* strain to protect 87% of vaccinated animals using a dosage of 3×10^8 to 3×10^9 Colony Forming Units (CFU). This was measured by official serologic tests and 83% for milk shedding and vaginal discharges. The RB51 vaccine demonstrated to be safe and innocuous when applied in pregnant small ruminant females; not abortion induction, milk shedding and vaginal discharges were observed (Martinez *et al*., 2003b). On the other hand, a unique specified dosage conferred protection at least for 36 months and immune response for more than 60 months (Bautista *et al*., 2003b; Molina *et al*., 2003), it was efficient because its use did a 3.4 : 1 benefit-cost relationship (Martinez *et al*., 2004b).

As a consequence of all the above mentioned, between December 2006 and October 2007, 4 vaccination operatives were conducted against caprine and ovine brucellosis using RB51 *Brucella abortus* vaccine. This procedure included 322 farmer that concentrated a mixed group of 5,168 animals (goats and sheep). The coverage of vaccination for the area was higher than 90% of the small ruminant herds located at Perote and Coffer Region, where,

brucellosis is an endemic disease. The studio considered the need to determine prevalence rate reduction impact at the communities above mentioned one year after of initiated the vaccination operatives.

MATERIALS AND METHODS

Study Area

This study took place at Frijol Colorado, La Gloria, Orilla del Monte, Tenextepec, Tlalconteno and Totalco communities, at Perote and Jalacingo Municipalities, in Veracruz State of Mexico. They were selected in order to had the higher goats and sheep inventory. Between, December 2006 and October 2007 more than 5,000 small ruminant females were vaccinated in four vaccination operatives.

Inclusion Criteria

In order to measure the impact provided by the vaccination operatives, a trial was conducted amongst animals of the above mentioned communities. Because, difficulties and expensiveness for sampling the total number of animals, a sample size was estimated in order to guarantee reliable results. Given the fact that it was not easy to determine if the females were vaccinated at the beginning of the operative, sample size was estimated considering unknown population and estimated prevalence with formula:

$$n = 1\text{-}p/(p)(v)$$

where, n is sample size, p is prevalence and v is variation coefficient.

Assuming that prevalence is unknown too, a considerated rate was 50% for selected communities and variation coefficient was 5% (Dohoo *et al*., 2003; Navarro, 1988; Smith, 2006); on that way, sample size (n) was 20 females per community, because feeding was done at the same pasture ground and water source; in other words, herds are mixed into the same community although, their owners are different.

Serology Diagnosis

Samples were obtained by jugular punction with sterile vacuum tubes system and without anticoagulant. Once the samples were collected, they were transported under refrigeration conditions at 4°C to an official authorized laboratory by SAGARPA at Boca de Rio, Municipality in Veracruz, State of Mexico to be processed. All collected sera were tested by 3% antigen concentration card test and the positive ones were confirmed by complement fixation test according with Mexican regulations (NOM-041-ZOO-1995, 1997).

Vaccination Procedures

All small ruminants females were vaccinated following procedures established by Mexican regulations (NOM-041-ZOO-1995, 1997) in order of edge at vaccination, applied dosage, specific considerations for authorized vaccines and special permits to use RB51 vaccine from SAGARPA state delegation and Veracruz State Government on goats and sheep according with previous results obtained at the selected zone (Martinez, 2002; Martinez *et al*., 2003a, b, 2005; Molina *et al*., 2003).

RESULTS

Samples were collected in two samplings in six different communities as it can be observed in Table 1. The number tested animals varied amongst the communities, as well as

Table 1: Number of serum samples collected by each community

Community	First sampling	Second sampling	Average
Orilla del Monte	26	16	21
Frijol Colorado	21	26	24
Tenextepec	22	18	20
Tlalconteno	20	20	20
La Gloria	13	28	21
Totalco	26	12	19
Total	104	120	112

Table 2: Brucellosis serum prevalence at selected communities obtained at the first sampling

Community	Vaccinated females	Herds	Serum prevalence (%)
Orilla del Monte	929	40	0.0
Frijol Colorado	536	16	0.5
Tenextepec	992	39	4.5
Tlalconteno	728	44	5.0
La Gloria	757	36	38.0
Totalco	86	7	1.4

Table 3: Brucellosis serum prevalence obtained by community at second sampling

Community	Vaccinated females	Herds	Serum prevalence (%)
Orilla del Monte	124	22	0.0
Frijol Colorado	282	30	0.0
Tenextepec	98	18	5.5
Tlalconteno	222	20	0.0
La Gloria	185	30	0.0
Totalco	229	20	0.0

between the first and second sampling. The biggest number of samples collected during the first sampling period corresponded to Orilla del Monte and Totalco communities, while in the second sampling La Gloria and Frijol Colorado were the communities with the higher number of tested animals. The total numbers animals studied during the first and second sampling was 104 and 120, respectively, with an average of 112.

During the first sampling, the serological studies of the samples showed no infection evidence for brucellosis in Orilla del Monte. In contrast, infection was demonstrated by these serological studies in all the other communities, with different seroprevalence as shown in Table 2. La Gloria had the higher ratio of seroprevalence (38%), while in the other 3 communities these ratio fluctuated between 0.5 to 5.

For the second sampling period, serum samples were obtained approximately 12 months after last vaccination operative and data are summarized in Table 3. All communities but Tenextepec were negatives to the presence of seropositive animals. The samples collected from Tenextepec showed a 5.5% seroreactors ratio.

DISCUSSION

Amongst the first and second samplings all females from selected herds at different communities were vaccinated and therefore, animal sampling at both times was randomly done targeting 20 serum samples per community approximately (Dohoo *et al*., 2003) in order to obtain significant results.

As it is shown in Table 2 herd inventory, herd quantity and serum prevalence differ between communities because, main economic activity and pasture ground availability were different; as an example Tenextepec and Tlalconteno communities are adjacent and their main activity is caprine production, but they are located at the most deforested zone as it happens in La Gloria community at Perote Valley and Coffer Regions (INEGI, 1991). This condition

represents a higher pressure directed to pasture grounds and according with different publications (Martinez *et al*., 2004a; Schurig, 1998; Stevens, 1997), brucellosis prevalence could be higher due to an inferior nutritional status. This is related with worse body condition and its influence for a higher risk to *Brucella* sp., infection.

Herd inventory, herd quantity and serum prevalence were different amongst communities during the second sampling procedure, as occurred during the first one (Table 3). This situation is related with the fact that vaccination operatives with RB51 vaccine must be done just once in the animals productive life, as it was demonstrated in previous studies (Franco, 2005; Molina *et al*., 2003) in which was established that just one vaccination could be enough to protect through all productive life in small ruminants populations. This fact also explains the decrease in vaccinated females, with the exception of Totalco community that could be vaccinated because they were young female replacements in order to be future breeders.

On the other hand, Table 2 and 3 showed how brucellosis serum prevalence was reduced in almost all the communities highlighting those at La Gloria where, serum prevalence rate went from 38% during the first sampling to 0% at the second one. These results are coincident with previous obtained (Martinez *et al*., 2003a, 2005; Suárez *et al*., 1998) in this sense that RB51 vaccine is very effective for *Brucella* sp., heterologous strains infection prevention.

Present study is very useful to support the fact that vaccination could be effective if a vaccination permanent program is established according with Mexican regulations (NOM-041-ZOO-1995, 1997), thus, in this way it is possible to avoid *Brucella* sp., field strains circulation, as it was proposed previously (Alton *et al*., 1988; Martinez *et al*., 2000, 2001b; Schurig, 1998; Stevens, 1997) resulting in an effective prevention of infection risk for another domestic and wild life mammals and of course for man that coexist with them or trough their products.

Respect to Tenextepec community a raise of 1% in prevalence rate was observed compared with the results obtained during the first sampling (Table 3); nevertheless, result could be explained in two ways; the first is that *Brucella melitensis* field strains remain at animal population as well as the infection possibility, although, previous studies conducted at the same community (Martinez *et al*., 2003a, 2004b) reported to obtain 87.5 and 90% efficacy rates, respectively. This means that observed serum prevalence was into the protection conferred rate. The second one is related with the nature of study, in which only one sample was obtained per each selected female, so the chosen animal was exposed to *Brucella melitensis* antigens and serumconverted; however, another studies (Cheville *et al*., 1993; Cheville *et al*., 1996; Molina *et al*., 2003), have demonstrated that animals protected by this vaccine, are exposed to *Brucella* sp. smooth strains as it occurs with *Brucella melitensis* field strains and develop serum conversion as a physiological response to challenge, but into 60 days period they could be desensitized and return as negative ones.

According with the amount of existing information related with RB51 vaccine obtained by several studies (Martinez *et al*., 2003b; Molina *et al*., 2003; Cheville *et al*., 1993; Schurig, 1998; Stevens, 1997), a common observation in all the studies is that postvaccinal effect is a clear evidence that conventional serologic test done by card and complement fixation tests employed in several countries as it happen in Mexico, does not represent any cross reaction difficulty as it occurs when animals have been vaccinated with traditional smooth strains Rev1 and S19 vaccines (Rodriguez, 1998) as it is shown in Table 2 and 3 for Orilla del Monte community, because at both samplings serum conversion was not observed. This observation means on one side that *Brucella* sp., field strains circulation could be very low and on the other, that small ruminants vaccinate with RB51 vaccine do not serum convert.

Finally and as a consequence of data shown in Table 3, an 80% serum prevalence rate reduction was observed at communities with any serum prevalence appreciated in Table 2 coinciding with previous observations (Martinez *et al*., 2003a, 2005) done at Tenextepec community and confirm that use of RB51 vaccine, in the dosage utilized for present study was effective to avoid serum conversion, safe and innocuous for vaccinated females.

CONCLUSIONS

Protective effect from RB51 *Brucella abortus* strain was demonstrated in five of six selected communities because reactors rate diminished with evident lower rate serum prevalence. On the other hand, 3×10^{8} to 3×10^{9} RB51 used dosage was very efficient to reduce 80% of brucellosis serum reactors in 12 months, did not induce serum conversion in sampled females as it could be clearly observed at Orilla del Monte community which confirms its safety and innocuousness.

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