

Field Experiences with RB51 *Brucella abortus* Vaccine in Goats from Tenex-tepec, Veracruz, Mexico

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Abstract

With the aim of evaluating the RB51 *Brucella abortus* strain under field conditions in a high goat brucellosis prevalence zone, a massive program of vaccination was made where RB51 *Brucella abortus* at 3×10^8 to 3×10^9 colony forming units (CFU) dosage was applied to a total of 1,489 greater goats of 3 months of age, from 83 different herds independently of their reproductive state. Also, to determine the brucellosis seroprevalence, 50 goats were sampled before and after vaccination, by means of the calculation as large as it shows based on a probabilistic model with unknown population and well-known prevalence of 40% ($n = 1 - p/pv$); where n = sample size, p = seroprevalence and v = coefficient of variation (0.05). Blood samples were obtained from all the goats in order to obtain serum samples, before and 180 days after vaccination. The serum samples of both sampling periods, were randomly selected and tested by conventional serological procedures of 3% card test (CT 3%), screening and complement fixation (CF) with 2 hemolytic units (UH) and 50% of haemolysis, as confirmative. 10% of goats sampled before vaccination, reacted as positive to CT 3% and 4% resulted positive to CF test; 6 months after vaccination, 10% also reacted to CT 3%, but only 2% were confirmed by CF test, which represents a 50% reduction in seroprevalence measured according the criteria established in NOM - 041 - ZOO - 1995 of Mexican regulations and an effectiveness of 80% in the prevention of seropositive ones. It is conclusive that the vaccination with RB51 *Brucella abortus* strain in goat herds from of Tenex-tepec, Municipality of Perote, Veracruz, Mexico is useful in the control of brucellosis.

Introduction

In Veracruz State, Mexico the prevention through the vaccination is a practice adopted by goat farmers since of 80's of the last century; nevertheless, it was until 1994 that settled down the systematic vaccination and obligatory as it was established by Mexican Federal Regulations (NOM) NOM-011-EM-ZOO-1994 and NOM-041-ZOO-1995, In times before these regulations, vaccination campaigns were contemplated by the old Federal Phitozoosanitary Health Law, but were not demanded for the commercialization and mobilization of farm animals (De Lucas, T.J. and Arbiza, A.S., 2001, Jimenez, D.B.M, 1994, Molina, S.B., *et al*, 2003).

For the Region of the Coffe and Valley of Perote, Veracruz, settled down a massive vaccination program contemplated for immunization of approximated 150 thousand heads of goats and 100 thousands of ovines. The program was planned to be executed in a 4 years (1994-1998) maximum period, in which they included both populations. At the beginning greater goats of 5 months of age immunized themselves; passed the time, females between 3 to 4 months of age, were immunized according with the NOM of reference (Martinez, H.D.I., *et al*, 2001a, Martinez, H.D.I., *et al* 2001b and Martinez, H.D.I., 2002).

Later to the vaccination program, prevalence studies for goat brucellosis were made, and results indicated that 80% of the animals behaved as reactors and eliminate, *Brucella melitensis* biovar-1 instead of being immunized. On that way, it was considered that homologous strain (*Brucella melitensis* strain Rev-1) used for vaccination females, did not demonstrate to help decreasing prevalence and incidence of brucellosis in human cases (Blasco, J.M., 1998, Mancera, M.A., *et al*, 1992, Martinez, H.D.I., *et al*, 2001a and Martinez, H.D.I., 2002).

The poor immune response / protection conferred by Rev-1 strain vaccination, forced to prove the protection obtained when using another type of vaccines as *Brucella abortus* RB51 strain, from which behaviour evaluated in terms of effectiveness, security and innocuous quality, possibility of revaccination of goats previously vaccinated with other strains and economic feasibility to be used in programs of goat vaccination (Al-Khalaf, S.A.S., *et al*, 1992, Blasco, J.M., 1998, Castell, B.H., 1998, Cheville, N.F., *et al*, 1993, Cheville, N.F., *et al*, 1996, Elzer, P.H., *et al*, 1999, Hernandez, A.L., 1999, Martinez, H.D.I., *et al*, 2001a).

Several recent research studies have been done evaluating the effectiveness of RB51 of *Brucella abortus* strain and concluded that dosage of 3×10^8 to 3×10^9 CFU, was the best to protect against brucellosis and to prevent elimination of field of *Brucella melitensis* biovar-1 strains. Effectiveness Using *Brucella abortus* RB51 strain, resulted in 87,5% of protection, measured through conventional serologic tests and 83,3% for

elimination of field *Brucella melitensis* biovar-1 strains the indicated dose (Bautista, B.R., *et al*, 2003a, Bautista, B.R., *et al*, 2003b, Martinez, H.D.I., *et al*, 2002, Molina, S.B., *et al*, 2003).

Another of the objectives using RB51 strain in goats, was to demonstrate that the proposed dose like Rev-1 *Brucella melitensis* strain was safe, since it was not shedding by milk of vaccinated animals; innocuous, because it did not cause abortion; and in animals already vaccinated with Rev-1 strain, It was also observed that animals revaccinated with RB51 strain, did not produce any seroconversion (Blasco, J.M., 1998, Mancera, M.A., *et al*, 1992, Martinez, H.D.I., *et al*, 2001a, Martinez, H.D.I., *et al*, 2001b and Martinez, H.D.I., *et al*, 2002).

Results of previous field trials demonstrated that RB51 *Brucella abortus* strain protects goats against the infection caused by *Brucella melitensis* biovar-1, under the same management conditions in Tenex-tepec community of Veracruz, Mexico, without any interference with the Mexican official conventional diagnostic tests such as card test (CT) and complement fixation (C'F).

Methods

During a vaccination operative against goat brucellosis at Tenex-tepec Community effectuated on march 2003, 1,489 goats older than 3 months of age without concerning their reproductive status, from 83 different flocks, were all vaccinated with RB51 of *Brucella abortus* strain using a 3×10^8 to 3×10^9 CFU dosage per female by the subcutaneous route at third mean of the neck of the right side.

In order to know the seroprevalence at the time of vaccination and after 6 months, a serum sample size was calculated to guarantee that the obtained results were significant and with a fair value of confidence, reason why the mathematical model based on not known population was used and considered prevalence, with the use of the following formula: $n = 1-p/(p)(v)$; where (n) is equal to sample size; (p) is the well-known prevalence of 40% for the zone and (v); the coefficient of variation with value of 5%. Thus, the resulting serum sample was at least 30. To guarantee the study 50 goats were sampled coming from 83 flocks, whose pasturing is made in communal lands, coexist with the other flocks in the same community although their owners differ.

All serum samples collected were randomly selected and put under two tests, one was the buffered acidified antigen at 3% concentration or 3% card test (3% CT), in agreement with Mexican regulation described by NOM 041 ZOO 1995; the positive ones, were submitted for second test by using C'F with 50 hemolytic units (HU) that was used as confirming one according with the same official criterion.

Results

During the first sampling, five (10%) samples were identified like 3% CT positive; nevertheless, when they were confirmed by means of C'F, only two (4%) were confirmed like that, as it can be observed in figure 1. For the second sampling from 50 tested females, 5 (10%) were positive to 3% CT; nevertheless, just one of them was confirmed as positive by means of C'F, which represents 2% of the total tested goats as it is pointed in figure 2. Both samplings could be resumed as seen in table 1.

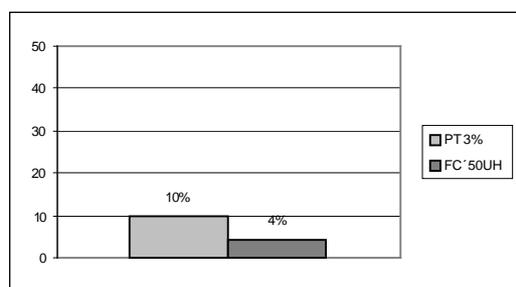


Figure 1. Seroprevalence during the first sampling

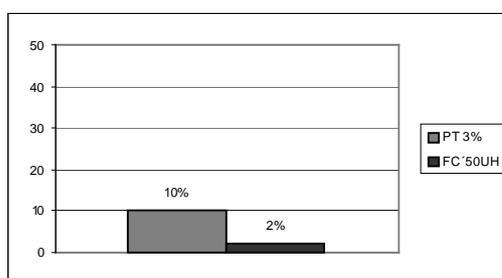


Figure 2. Seroprevalence during the second sampling

Table 1 Brucellosis Goat Seroprevalence at Tenex-tepec, (%)

Sampling	3% CT Positives	C'F Confirmed
First	5(10)	2(4)
Second	5(10)	1(2)

Discussion

3% CT, is used in Mexico to test goat and ovine serums from herds whose epidemiologic condition is unknown or in those where they have passed more than 60 days in which the animals were put to the serologic diagnostic test for brucellosis, because its high sensitivity, reason why 3% CT is considered as screening test (Alton, G.G., *et al*, 1976, Angus, R.D. and Barton, C.E., 1984, Blasco, J.M., 1998, Juarez, S.M.E., 2002, NOM-011-EM-ZOO-1994, NOM-041-ZOO-1995).

C'F is the test used in Mexico for confirmation 3% CT goat and ovine positive serums (Angus, R.D. and Barton, C.E., 1984, Diaz, A.E., *et al*, 1994, Diaz, A.E., *et al*, 2001), because of the, results obtained in this study showed that the goats of Tenex-tepec vaccinated with *Brucella abortus* RB51 strain were exposed to similar antigens to those of *Brucella* spp; nevertheless, since the IgG2 is identified in abundant amount in the chronic brucellosis, C'F demonstrated that 80% of 3% CT positive females, were negative to this one (figure 2), which agrees with observations of another works did in Mexico (Martinez, H.D.I., 2002, Suarez, G.F., *et al*, 1998) in the sense that RB51 strain protects against the serum conversion induced by smooth *Brucella* spp strains and therefore against the infection, as identifies in figure 1 when comparing the results of both samplings.

According with existing information (Diaz, A.E., *et al*, 2001, Juarez, S.M.E., 2002, Martinez, H.D.I., *et al*, 2003, Suarez, G.F., *et al*, 1998, Stevens, M.G., *et al*, 1997), RB51 strain showed similar results given that postvaccinal effect did not influence results of conventional serologic diagnostic tests such as 3% CT and C'F used in Mexico for small ruminants, These results can be understood that animals vaccinated with RB51 strain are exposed to *Brucella* spp smooth antigens, are able to produce serum antibodies; however, more than 80% of them will do it only with IgM high concentrations, without the risk in which the infection becomes chronicle and represents a public health risk and the one of the vaccinated animals, since another study did at Tenex-tepec (Molina, S.B., *et al*, 2003), has demonstrated that RB51 protects at least by 36 months.

An additional complementary information is that animals vaccinated with *Brucella abortus* RB51 strain that serum convert to 3% CT and could be confirmed by C'F, could be considered like positive ones, without diagnostic interference due to vaccination (Diaz, A.E., *et al*, 1994, Diaz, A.E., *et al*, 2001, Diaz, A.E., *et al*, 2003, Martinez, H.D.I., 2002), which allows to advance in establishing campaign programs contemplated in Mexican regulations (NOM-041-ZOO-1995).

Another observed effects by vaccination with *Brucella abortus* RB51 strain in ovines (Martinez, H.D.I., *et al*, 2001a, Martinez, H.D.I., *et al*, 2001b, Martinez, H.D.I., *et al*, 2002, Martinez, H.D.I., 2002, Suarez, G.F., *et al*, 1998) besides to see a favorable effectiveness against serum conversion, as it happens in this field trial and others (Castell, B.H., 1998, Hernandez, A.L., 1999, Mancera, M.A., *et al*, 1992) that they have evaluated innocuous, security and null serum convert in goats vaccinated with *Brucella melitensis* strain Rev-1 and then revaccinated with RB51; also, have been observed other important productive data like the fertility index, rate of mortality in lambs within the first 30 days of life and the rate of herd recovery in affected ones by smooth *Brucella* spp strains, where the RB51 always has obtained very encouraging results (Elzer, P.H., *et al*, 1999, Jimenez, D.B.M., *et al*, 1994). Thus, during the six months passed between animal vaccination and the serologic evaluation of the second sampling, abortions, problems of infertility or elimination of field strains at Tenex-tepec have not been registered.

The results observed in these field trials are in agreement with several of studies made Mexico by other researchers in the sense that *Brucella abortus* RB51 strain does not induce abortion in pregnant females and differ from the observations made in another one (Diaz, A.E., *et al*, 2003, Juarez, S.M.E., 2002, Leal, H.M., *et al*, 2003), where herds infected with *Brucella melitensis* biovar 1 to which were vaccinated with *Brucella melitensis* Rev-1, *Brucella abortus* RB51 or stayed like controls without vaccinating, they did not show significant differences between the vaccinated RB51 ones and controls; nevertheless, the dosage used and the source of reference of RB51 strain, also are different from which has been used in this last work (Diaz, A.E., *et al*, 2003, Hernandez, A.L., 1999, Mancera, M.A., *et al*, 2002, Martinez, H.D.I. *et al*, 2001a).

Conclusions

1. *Brucella abortus* RB51 strain in dose of 3×10^8 to 3×10^9 CFU reduced 50% the initial seroprevalence of goat brucellosis at Tenex-tepec, Veracruz, Mexico.
2. *Brucella abortus* RB51 strain effectiveness in the prevention of serum convert by smooth *Brucella* spp strains at Tenex-tepec, Veracruz, Mexico goats, was of 80%

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