Effectiveness of *Brucella abortus* RB51 and S19 Strains to Reduce Abortion Rate


1CE La Posta-INIFAP, Km 22.4 Carretera Veracruz-Córdoba, Paso del Toro, Col. AP. 898 Suc. A, C.P. 91700, Veracruz, Veracruz, México
2Facultad de Medicina Veterinaria y Zootecnia (FMVZ), Universidad Veracruzana (UV), Circunvalación Esq. Yañez S/N, Col. Unidad Veracruzana, C.P. 91710, Veracruz, Veracruz, México
3CENID Microbiología Animal-INIFAP, Km 15.5 Carretera México-Toluca, Col. Palo Alto, C.P. 05110 México, D.F.

**Abstract:** To determine *Brucella abortus* RB51 and S19 strains effectiveness to prevent abortion, two dual purpose cattle production units (PU) infected with brucellosis were selected at Veracruz, Mexico with tropical climate. Each PU had at least 200 cows. At both locations, cows were randomly selected and divided into two groups, one half was vaccinated and the other one remained unvaccinated as a control group. Abortion rate was calculated dividing total abortions by total births. The PU health status related with brucellosis was established according to Mexican regulations and by bacteriological studies according to WHO procedures. Results were analyzed by Xi² and association by relative risk (RR). Vaccine effectiveness was calculated by

\[
VE = \frac{CDR-VDR}{CDR} \times 100
\]

where: \(VE\) = Vaccination efficacy; \(CDR\) = sick animal rate within the control group and \(VDR\) = sick animal rate within the vaccinated group. This study was performed in 18 months, divided into six quarterly surveys and then integrated into two nine months periods. At PU vaccinated with S19, the abortion rate in the vaccinated group was 0.76% and control 0.89% in the first post-vaccination period. In the second one, the vaccinated group had no abortions and the control increased to 1.9%; however there was not relationship between abortions and vaccination (\(p>0.05, RR=0; CI_{95\%}:0-0\)), for an efficacy of 100%. For PU vaccinated with RB51, there were no abortions in the two groups during the first period, for the second one, there were only 2.8% abortions in the control group, so there was not relationship between abortions and vaccination (\(p>0.05, RR=0; CI_{95\%}:0-0\)) and effectiveness was 100%. In conclusion, both vaccines are safety because they do not induce abortion and are effective in abortion prevention at PU infected in Mexican tropic.

**Key words:** Abortion rate % Production unit % Vaccines safety

**INTRODUCTION**

In Mexico, livestock activity suffered a significant setback, particularly at the tropics in dual purpose systems because of its limitations in adopting leading edge technologies to benefit production by keeping sanitary conditions, as well as good reproductive management of herds. However, main difficulties that could be found are infectious diseases, metabolic or poor production practices that lead to abortion, which in addition to discouraging producers, represent a significant economic impact for their farms [1].

From a reproductive point of view, the main economic losses are due to brucellosis and many other reproductive diseases because of the abortions at any stage of pregnancy; although, they are more evident at the last quarter of gestation [3]. Major effects of abortion are fetal loss, retained placenta and metritis in affected cows, as a result uterine recovery could be very low and consequently ovary activity do not restart and open days will increase [2].

In dual purpose systems, animals are fed in an extensive system. The cows are on pasture all the time and usually they are not watched closely until they calved.
and start lactating. This type of management makes very difficult to detect the abortions. In addition, the farmers seldom register calving dates, so abortions could pass undetected and therefore, less milking cows and less calves are produced every year [1].

Also, under dual purpose production systems, milk is used to pay direct costs at PU, whereas the calves are sold for future investments or major expenses, thus brucellosis, due to calve losses, is a disease that has an impact on the possibility of adopting technologies that require investing [1].

On the other hand, Mexican official regulation [3] establishes that for control and eradication, vaccination against brucellosis is mandatory, since it has been proven to be an easy and economic way to prevent abortion and disease dissemination through animal population [4,5]. Thus, the aim of the present study is to identify if there is a benefit in vaccinating with RB51 and S19 Brucella abortus strains to reduce abortion rate at persistently infected herds from Veracruz State tropic zone.

MATERIAL AND METHODS

Study Site: The study was carried out at “El Desengaño” community in Las Choapas, Municipality of Veracruz, Mexico from August 2006 to February 2008. A Cross sectional epidemiological study was carried out at the beginning of the research in order to identify herds that were naturally infected with brucellosis.

Inclusion Criteria: Only PU devoted to dual purpose production in an extensive system in a tropical climate and without brucellosis vaccination were included in the study. All animals six month-old and older were sampled according to NOM-041-ZOO-1995 “National Campaign against Brucellosis in Animals” [3]. An infected herd was defined as the one where there were animal reactors to card test (CT) with 8% concentration antigen with at least one positive case confirmed by rivanol test (RT).

Serology Diagnosis: Blood samples were taken from the coccygeal vein using vacuum tubes without anticoagulant. Then, they were transported in refrigeration at 4°C to the Microbiology laboratory of the Faculty of Veterinary Medicine of Universidad Veracruzana. Serum was separated and placed in previously identified vials with sample number and preserved at-20°C until processing by the CENID Laboratory-Animal Microbiology of INIFAP at Mexico city, by CT and RT according with NOM-041-ZOO-1995.

Clinical Assay: According to inclusion criteria, for clinical assay, a herd infected with brucellosis was selected. In order to estimate sample size and establish the vaccinated and non-vaccinated groups, Win Episcope 2.0 program was used under the modality of “finding difference between proportions” by estimating an expected proportion of 6% of brucellosis positive animals in vaccinated population and 20% positive animals in the non-vaccinated population, with a level of confidence of 95% and a potency of 80%. Thus the sample size was estimated at 88 animals per group [6]. Vaccinated and non-vaccinated sexually mature females groups were randomly selected at every PU and identified by ear tags.

Vaccination: Cows that had negative results to CT and RT were integrated into the vaccinated group and then vaccinated subcutaneously once, at the mid third of the neck on the left side. Strains RB51 or S19 vaccines were used in doses of 3 X 10^8 to 3 X 10^9 CFU in selected PU respectively, including pregnant cows according to Mexican regulations established in NOM-041-ZOO-1995 [3]. Vaccination of females at respective PU was carried out in August 2006.

Pregnancy Diagnosis: To estimate abortion rate every trimester starting at vaccination date, pregnancy was determined by rectal palpation. Findings such as metritis and/or placental retention that could explain abortion were registered. If a cow was detected with placental retention or metritis, placental tissue samples or swabs from vaginal exudates or uterine discharges were taken for bacteriological and serological diagnosis. Also, every trimester reproductive records were checked to compare pregnancy rates, parturition, abortions, service date, due date and early pregnancy or any clinical evidence that could be useful to identify abortion into each female group at selected PU.

Abortion Rate: The information from each trimester was concentrated into two periods of nine months since the gestation period in bovine is very similar (about nine months) in order that pregnancy period in the cow is very similar, to ensure that a cow could be pregnant at least once in 18 months. Thus abortion rate including previously mentioned criterion was estimated by the number of abortions divided by the number of total births in the same period.
Statistical Analysis: To determine abortion rate, Relative Risk (RR) and Confidence Intervals (CI) of 95% were estimated according to Thrusfield [7] and statistical significance of observed abortion rates in vaccinated and non-vaccinated groups was estimated by chi-square. Significant differences were considered when p<0.05 [7].

Vaccination Efficacy: It was estimated by the formula VE= CDR-VDR / CDR x 100, where: VE= Vaccination efficacy; CDR = sick animal rate within the control group and VDR = sick animal rate within the vaccinated group [8].

RESULTS

Abortion Rate for PU Vaccinated with S19 Vaccine:
Serological rates for vaccinated and non vaccinated groups were 0.76% and 5.3%, respectively for the first 9 month period. Abortion rate in the vaccinated group was 0.76%, because from 131 pregnant cows only one abortion was detected, however, this cow was healthy according to bacteriological and serological tests for brucellosis, so it is possible that the cause of abortion was not this disease and allows us to suspect of another infectious agent, metabolic disorder or poor reproductive management. On the other hand, in the non vaccinated group, 112 females were pregnant and also only one abortion was observed for a 0.89% abortion rate. Even though serological CT and RIV tests identify the cow as negative, Brucella abortus was isolated from uterine discharges collected by bacteriological procedures.

On the other side, because abortion rate was very low for both groups at the first period, vaccination with S19 strain was not related with the abortion observed in the vaccinated group (p>0.05; RR = 0.92; CI_{95%}: 0.024-3.53), as it could be seen in table 1. In the same period VE was 14.6% since abortion rates for both groups were almost the same; however, abortion differences lies in the fact that in the vaccinated group, the cause of abortion could be other than brucellosis, but not for non vaccinated as it was demonstrated by bacteriological procedures.

For the second period serological rates for vaccinated and non vaccinated groups were the same as the first one, however, in the vaccinated group there were not abortions reported from 109 pregnant females, thus, the assessment that there was not relationship between abortions and vaccination was maintained (p>0.05; RR = 0; CI_{95%}: 0-0). On the other hand, in the non vaccinated group, abortion rate increased from 0.89% to 1.9% (Table 2), nevertheless, the females that aborted were also negative for serological and bacteriological diagnostics

tests. Again as it was mentioned for the first period, abortions could be other than brucellosis. For this period VE was 100% but only apparently because none of the abortions were due to brucellosis; however, since we found no relationship between abortion rate and vaccination (RR = 0; CI_{95%}: 0-0), one could imply that vaccine conferred herd immunity with protective effect against abortion.

Abortion Rate for PU Vaccinated with RB51 Vaccine:
During the first period serologic rates for vaccinated and non vaccinated groups were 0% and 15.71%, respectively. It was possible to identify 96 pregnant females for the vaccinated group and 70 pregnant females from the non vaccinated group without abortions as it can be observed in table 3. It should be noted that although the presented observations did not show differences between groups (p>0.05), for abortions and vaccination, it is also important to say that RB51 vaccine did not induce abortion in any of the vaccinated pregnant females (RR = 0.87; CI_{95%}: 0.87-0.87). On the other side, using the obtained values it was not possible to estimate VE.

Table 1: Abortion and serological rates (%) at PU vaccinated with S19

<table>
<thead>
<tr>
<th>First period</th>
<th>Abortion</th>
<th>Pregnant</th>
<th>Serum reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated Group</td>
<td>1(0.76)</td>
<td>131</td>
<td>0(0.76)</td>
</tr>
<tr>
<td>Non Vaccinated Group</td>
<td>1(0.89)</td>
<td>112</td>
<td>6(5.3)</td>
</tr>
</tbody>
</table>

*p=0.05, 95% CI = 0-0.87

Table 2: Abortion and serological rates (%) at PU vaccinated with S19

<table>
<thead>
<tr>
<th>Second period</th>
<th>Aborted</th>
<th>Pregnant</th>
<th>Serum reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated Group</td>
<td>0</td>
<td>109</td>
<td>1(0.76)</td>
</tr>
<tr>
<td>Non Vaccinated Group</td>
<td>2(1.9)</td>
<td>105</td>
<td>6(5.3)</td>
</tr>
</tbody>
</table>

*p=0.05, 95% CI = 0-0.92

Table 3: Abortion and serological rates (%) at PU vaccinated with RB51

<table>
<thead>
<tr>
<th>First period</th>
<th>Abortion</th>
<th>Pregnant</th>
<th>Serum reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated Group</td>
<td>0</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td>Non Vaccinated Group</td>
<td>0</td>
<td>70</td>
<td>1(15.71)</td>
</tr>
</tbody>
</table>

*p=0.05, 95% CI = 0-0.87

Table 4: Abortion and serologic rates (%) at PU vaccinated with RB51

<table>
<thead>
<tr>
<th>First period</th>
<th>Abortion</th>
<th>Pregnant</th>
<th>Serum reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated Group</td>
<td>0</td>
<td>79</td>
<td>0</td>
</tr>
<tr>
<td>Non Vaccinated Group</td>
<td>2(2.8)</td>
<td>71</td>
<td>1(15.49)</td>
</tr>
</tbody>
</table>

*p=0.05, 95% CI = 0-0.87
For the second period, serologic rates were also the same as the first one. Abortion rate for the vaccinated group was 0% because from 79 pregnant females non aborted and for non vaccinated one, two abortions were observed for 2.8% abortion rate (Table 4); however, aborted females remained negative for brucellosis according to serological and bacteriological diagnostics tests. Also, VE was 100% and no differences (p>0.05), were observed between groups, nor association between abortion and vaccination, so there was a protective effect conferred by RB51 vaccine for abortion (RR = 0; CI95: 0-0).

DISCUSSION

Abortion Rate for PU Vaccinated with S19 Vaccine: There is evidence [9] that aborted females could be negative to diagnostic serological tests despite being infected with *Brucella abortus* due to vertical transmission [2]; thus if a fetus is infected during the first half of gestation or if a female is persistently infected, immune system could recognize as their own bacterial epitopes that in case of calving, the new born animal will never be able to recognize as strange, so that if this animal is a female and gets pregnant, it could abort and not be diagnosed by conventional serologic tests [10,11]. This could have happened with one female during the first sampling in our study.

These kind of animals are known as tolerant ones [3,6], represent a serious public and animal health problem, an economic impact factor for PU affected, particularly at extensive production systems because they can maintain a silent infection in the herd and are almost impossible to detect without productive records or good reproductive management practices [12].

On the other side, it has been noted [13,14] that S19 strain vaccination induces abortion in almost 1% of vaccinated females, particularly during the last trimester of gestation. However, although an abortion was observed in the first period in the vaccinated group, bacteriological procedures performed in uterine discharges and statistical analysis (p>0.05), together with RR evaluation (RR = 0.92; CI95: 0.024-3.53), demonstrated that vaccine strain was not the cause of abortion and that S19 vaccine is safe to be used in bovines at the tropics. Also, VE is apparently very low (14.6%) and obviously, the preventive effect against abortion was poor, but it is also true, that none of vaccinated females aborted for this cause as it had been described by various authors [15-17].

It is also well known that vaccination against brucellosis at infected herds stop the abortion occurrence [15,17]. In fact it does so in a much more efficient way than the way it protects against infection that generate measurable antibodies by conventional official serologic tests (serum conversion), as it happens with CT and RIV [3]. Tables 1 and 2 show that, while abortion rate was 0.76% during the first nine months, vaccination reduced it to 0% for the second nine months. This did not happen in the non vaccinated group because abortion rate increased from 0.89% to 1.9%. Protective effect against abortion was reinforced with RR obtained parameter (RR = 0; CI95: 0-0), which agrees with Thrusfield and Toma [23,26] which noted that a risk value under 1 and a CI95 also below 1 is due to the variable under study.

Abortion Rate for PU Vaccinated with RB51 Vaccine: Unlike Van Metre [18], who reported an infected pregnant female with RB51 strain previously vaccinated with the mentioned vaccine strain, in our study none of the females belonging to vaccinated group aborted nor were infected with brucellosis. This agrees with Edmonds and Olsen [19; 20], who conclude that RB51 vaccine did not induce abortion or reproductive problems when it was applied to sexually mature or pregnant females. Also, when vaccination was applied to females into the last trimester at a dose of 3 X 10⁹ CFU as it was done in this study, Uza [21], considered RB51 as a safe and very useful vaccine because neither produced diagnostic interference measured with serologic conventional tests and nor induced abortion.

On the other side, according with Casas and Stevens [16,22], vaccination with RB51 strain reduced the infection rate because induced a strong protection against brucellosis just three to four weeks after it was applied and also, it is useful to decrease exposition to *Brucella abortus* field strains from infected animals at affected PU. This could explain the reason of differences between serum reaction rates in the vaccinated and control groups (Table 3).

Leal [23] indicated that RB51 vaccine protected 94% of females in the herd against brucellosis at endemic zones; however, 100% of VE obtained in females from vaccinated group was very similar and agrees with the findings observed by Lord and Ramirez [24, 25], who demonstrated that this strain did not induce serum conversion nor abortion and protected the 100% of susceptible livestock when it was used to control brucellosis at PU with low prevalence.
CONCLUSION

S19 and RB51 strains protected to vaccinated females against abortion and were safe because they did not induce abortion by themselves; nevertheless, protective effect against abortion for RB51 strain was observed from the beginning after application and for S19 strain the effect was perceptible after a 9 months period. VE against abortion for both vaccines was 100% after 18 months of observation.

ACKNOWLEDGEMENTS

This study is part of the requirements that the first author must cover to obtain the degree of Master in Animal Sciences granted by the Universidad Veracruzana. Research received support and financing from the project “Comparative study of strain RB51 and strain S19 efficacy in the prevention of brucellosis in herds with different sanitary conditions” of the National Forestry, Agriculture and Livestock Research Institute (INIFAP) called for by SAGARPA-CONACYT 2004 Sector fund 23.

REFERENCES

13. Davis, D.S. and P.H. Elzer, 2002. Brucella Vaccines In Wildlife; Departaments Of Veterinary Pathobiology And Wildlife And Fisheries Sciences, Texas A Y M University, College Station, TX, USA, Department of Veterinary Science, LSU Ag Center, Baron Rouge, LA, USA, Veterinary Microbiology. Science Direct, Veterinary Microbiology Volume 90(1-4): 533-544


