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Morpho Metrical Evaluation of Preescapular Lymph Nodes from Cattle Vaccinated with *Brucella abortus* Strains S19 or RB51

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Abstract: With the aim of measuring preescapular lymph node response in bovines vaccinated with either S19 or RB51 *Brucella abortus* strains vaccines, two herds were selected. About 40 females were divided in two groups of 20; group 1 was vaccinated and group 2 left as unvaccinated control. Vaccinated group, 3 and 6 months females were vaccinated with 5×10^{10} Colony Forming Units (CFU) dosage and >6 with a 3×10^{8}-3×10^{9} S19 strain. For the second herd same age females and dosage was used but applying RB51 strain. Vaccines were subcutaneously applied at mid third on left side of the neck. Preescapular lymph nodes were measured in length and wide at 90 and 180 days after vaccination. Results were analyzed by Chi-square. At 90 days post vaccine, lymph nodes dimensions were 3.7-4.8 cm wide×10-11.7 cm length and for unvaccinated 3.8-4.5×10-11.5 cm. No statistical differences were found ($p>0.05$) among vaccinated and unvaccinated controls. After 180 days post vaccine similar results were found with RB51 for both groups but strain 19 vaccinated herd was different ($p<0.05$) however, differences were found in control group. Results indicate that both strains are efficiently processed by lymphatic tissue among 90 days after vaccination and therefore, they may be considered as safe without producing macroscopic changes that alter organ dimensions.

Key words: Brucellosis, lymphatic tissue, dosage, dimension, safe, macroscopic

INTRODUCTION

Brucellosis is a zoonoses affecting domestic and wild mammals mainly bovines, ovines, caprines and swine however, under some circumstances the infection can occur in humans but there is not a natural mechanism through infection can be established in man (Munoz *et al*., 2005).

According with the World Health Organization (WHO), brucellosis is considered the most important zoonoses transmitted from animals to man. Consequences in affected people varies from a slight fever to serious complaints that can cause death. In animals, brucellosis causes significant economic losses, affecting productive parameters such as fertility, stillbirths, low parturition rates, milk yield, abortions increase in the open day interval, orchitis and others (Smits and Kadri, 2005). Changes in eating habits, mainly with pasteurized milk and their products can help to prevent the disease in animals and man.

Otherwise, brucellosis in animals can be prevented by vaccination. There are three well-known vaccines, two strains for *Brucella abortus* (S19 and RB51) and one for *Brucella melitensis*, strain Rev-1; the last one is used in ovines and caprines.

RB51 strain has an advantage over S19 given by the lack of O lipopolysacaride lateral chain from *Brucella* sp. smooth types characterized by not producing antibodies that could interfere with conventional serological tests therefore, never exposed herds to smooth *Brucella* sp. strains vaccinated with RB51 strain, results negative in all conventional tests (Stevens *et al*., 1997). Studies realized in United States of America and some other American continent countries such as Mexico have shown similar

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abortion and infection protection than strain 19. Strain S19 is used in Mexico to protect bovine herds from brucellosis since, 40's decade and today as a mandatory law given by NOM-041-ZOO-1995 however, a main disadvantage is its interference with conventional serologic tests. The classical vaccine dose is used for 3-6 months old females and the lower dose of vaccine is used for older animals including pregnant. The immunity provided by the strain confers protection rate around 70% with <1% abortion.

When a vaccine is applied, antigens are transported to local lymph nodes by antigen presented cells which will react against antigen presence in order to eliminate it lately (Abbas *et al*., 1994; Tizard, 2004). In consequence lymph nodes where antigen presence exists as it occurs after vaccination, their size can be modified several times (Tizard, 2004).

Animals immunized with 19 or RB51 strains, develop resistance mechanisms against disease among 3-4 weeks after vaccination. The most important are related with the presence of activated macrophages and gamma interferon production (γIFN) which are mediated by T Lymphatic helper cells (TLh) or CD4+. TLh are classified into two main types, Th1 or Th2 depending on the immunity response that they could activate. Th1 produces IL2, γIFN and FNTβ; Th2 are cooperative cells that activate B lymphatic cells resulting in antibodies production as well as IL-4, IL-5, IL-6 and IL-10 production (Baldwin, 2002; Gorvel and Moreno, 2002).

As it occurs in other intracellular facultative bacteria, *Brucella abortus* expresses high protein levels as a result of multiple stimuli from the intracellular medium if it is exposed to high temperatures or acid conditions (De Bruyn *et al*., 2000).

Brucella sp. has proteins of GroEL family that are of thermal shock similar to *Escherichia coli* and *Mycobaterium* sp. (Vemulapalli *et al*., 2002). These proteins can induce to humoral and cellular immune responses which may cause an increase in bactericidal activity from macrophages resulting of cytokines production like γIFN and FNTβ (Gross *et al*., 1998; Baldwin, 2002).

Vaccinated animals with any strain vaccines develop as a first response lymph adeno megalic reaction at lymph nodes close to application zone. However, 3-4 weeks after any vaccine is applied (Baldwin and Parent, 2002), both are efficiently processed by lymphoid system as a result it is almost impossible to isolate them from any body tissues and a consecutive process for normal physiological delay for initial recovery to a normal size for the referenced nodes. On the other hand this important physical aspect has lack of meaning for farmers and the main reason of the present research is to identify the time elapsed between vaccine and desensitization of vaccinated animals which means a correct response for protection in vaccinated animals against natural infection by field strains.

MATERIALS AND METHODS

At the community El Desengano in Las Choapas at Veracruz state of Mexico a clinical assay was carried out in order to evaluate RB51 and 19 *Brucella abortus* strains as vaccines with the purpose of determining general efficacy on infected bovine herds in the Mexican tropic. Las Choapas, Ver. is located in the coordinates North latitude 17°55' and West latitude 94°06', 10 m above the sea level with an annual average temperature of 27°C and a pluvial precipitation of 2,900 mm year^{-1}.

Two independent farms with an active infection caused by *Brucella abortus* were selected for present study. In one of them a half of females were vaccinated with strain S19 and the other one was not vaccinated to be used as control group. The other farm was vaccinated with RB51 according the same criterion than the S19 vaccinated farm. From each farm 40 females were randomly selected in order to obtain 50% of the animals vaccinated and the other ones not.

Sample size was determinated using Win Episcope Ver. 2.0 software (Thrusfield *et al*., 2001) to determine exposed animals from a population of 200 animals where 50% of females were vaccinated with 95% of confidence resulting in a sample of five females per group. However in order to develop a clinical assay and allow to estimate significance and association measurements, sample size was increased to 20 animals per group.

Both strains vaccines were elaborated by Nova Litton de Mexico, S.A. de C.V. laboratory. Each strain was applied by subcutaneous via on the third half of the neck at the left side according to Mexican regulations (NOM-041-Z00-1995) as it is shown in Table 1. After vaccine application a measurement of preescapular lymph nodes was done on the neck of the females of the vaccinated groups. For this purpose, a vernier was used to register bilaterally the dimension in length and wide of each node as shown in Fig. 1 and 2. After vaccination, measurements were done in two different times, 90 and 180 days after vaccination.

Table 1: Vaccine dosage used for the morphometrical evaluation of preescapular lymph nodes from bovines vaccinated with either strain S19 or RB51 *Brucella abortus* vaccines

Strain	Normal dose	Reduced dose
S19	3-6 months: 5×10^{10} CFU	Older than 6 months: 3×10^{8} a 3×10^{9} CFU
RB51	3-12 months: 5×10^{10} CFU	Older than 12 months: 3×10^{8} a 3×10^{9} CFU

[a]CFU = Colony Forming Units

Fig. 1: Preescapular lymph node length

Fig. 2: Preescapular lymph node wide

Results from measurements were analyzed for significance by Chi square (χ_i^2) using Win Episcope 2.0 (Thrusfield *et al*., 2001).

RESULTS AND DISCUSSION

Preescapular lymph nodes dimensions were obtained measuring length and wide from all selected animals, the ranges between both measurements were calculated through standard deviation. Measurements from vaccinated and non-vaccinated females at 90 and 180 days after the vaccination allowed to find out dimensions from 3.7-4.8 cm wide per 10-11.7 cm of length and of 3.8-4.5 cm wide per 10-11.5 of length each, respectively. This means that despite of the vaccination with *Brucella abortus* RB51 or S19 strains from 90th day after the vaccination there is no difference ($p>0.05$) between the evaluated dimensions of the lymph nodes of the vaccinated and non-vaccinated animals. In order to establish a different dimensions between length and wide for evaluated lymph nodes, any animal that showed an equal or higher increase obtained by the mean value plus one standard deviation of any measurement was been considered as a reactor.

Table 2: First morphometrical evaluation 90 days after vaccination with strain RB51 vaccine

Treatments	Reactors	Negatives	Total
Vaccinated	7[a]	23[a]	30
Controls	8[a]	20[a]	28
Total	15	43	58

Table 3: Second morphometrical evaluation 180 days after vaccination with strain RB51 vaccine

Treatments	Reactors	Negatives	Total
Vaccinated	6[a]	20[a]	26
Controls	6[a]	21[a]	27
Total	12	41	53

Table 4: First morphometrical evaluation 90 days after vaccination with strain 19 vaccine

Treatment	Reactors	Negatives	Total
Vaccinated	10[a]	20[a]	30
Controls	6[a]	23[a]	29
Total	16	43	59

[a]Equal letters between lines do not show significant differences ($p>0.05$)

During the first measurement corresponding to the 1st 90 days after vaccination with RB51 strain, difference ($p>0.05$) between both groups as it is shown in Table 2. Second measurement (180 days after the vaccination with RB51 strain), six reactor animals from the vaccinated group and six from the non-vaccinated group were observed which also means that any difference was observed between groups as it is shown in Table 3.

On the other hand, for the first measurement in the lymph nodes from farms with *Brucella abortus* S19 strain was observed that measurement done at 90th day after vaccination, 10 females from the vaccinated group and six from the non-vaccinated group were reactors and their mean values were also non significantly different as it is shown in Table 4. At 180 days after the vaccination with S19 strain, three reactor animals from the vaccinated group and 8 from the non-vaccinated one as it is shown in Table 5 let us observe significant differences between groups ($p<0.05$).

Dimensions of preescapular lymph nodes from bovines go between 1×3.5, 2.5×10-13 and 2×1-10 cm (Jackson and Cockcroft, 2002) this differs from the observed in this study which means that maybe for dimension estimation age and the breed from used animals were not considered. However, Luna and Mejia in a postmortem inspection related to verify carcass for identification of macroscopic wounds caused by bovine tuberculosis in Mexico show that node size varies from 3×7-10 cm which agree with the results obtained at present study. This means that measurement findings are common to animals with similar age to the animals used in this study and is also similar for most of the breeds that are slaughtered in the country.

Table 5: Second morphometrical evaluation 180 days after vaccination with strain 19 vaccine

Treatments	Reactors	Negatives	Total
Vaccinated	3[a]	19[a]	22
Controls	8[b]	11[a]	19
Total	11	30	41

[a]Equal letters between lines do not show significant differences ($p>0.05$)

Results shows in Table 2 concur with those reported by Bautista-Beranza were it was established that persistence of vaccines strains in lymph tissue on vaccinated caprines with *Brucella abortus* RB51 strain are processed in the correct way by sensitive macrophages and Th lymphocytes during the 1st 60 days after vaccination. Furthermore, other investigation (Baldwin and Parent, 2002) shows that immunized animals with *Brucella abortus* S19 or RB51 vaccine strains develop resistance mechanisms against the disease between 3-4 weeks after being vaccinated which allows elimination of source of stimuli this means that lymphatic tissue returns to its original dimensions.

In mice and bovine infection models (Cheville *et al*., 1992; Winter *et al*., 1996; Olsen *et al*., 1998; Baldwin and Parent, 2002) vaccine strains are processed in the lymphoid tissue by Th1 and the activated macrophages until its elimination is achieved as a result the clone of Th1 memory cells can be present forever and will induce immunity against the pathogenic strains (Palmer *et al*., 1996; Baldwin, 2002). Present results have shown that animals develop an immunological reaction resulting in satisfactory protection level.

Therefore, vaccinated animals develop an immunological memory without interfering with the physical dimensions by vaccination but helps to solve future exposures. Studies done in mice BALB/c (Stevens *et al*., 1994a, b) in which immune and pathological responses of lymphocytes proliferation during 20 weeks exposed to the *Brucella abortus* strains 19, 2308 and RB51 were evaluated. Results demonstrated that RB51 strain is processed during the 4 weeks after the infection while in the strain 19 it is developed during the week 6 while in the strain 2308 occurs during the 10th week. This data concurs with the rise in dimensions of the lymph nodes of the six vaccinated animals (Table 3) in the present study and proves that it was not caused by the antigenic stimuli produced by the strain RB51. This is proof by the fact that in control group exists an equal number of members considerate as reactors despite of not having received the vaccination with RB51.

Results obtained 90 days after vaccination using S19 strain are very similar to the observed results with the strain RB51 as it is shown in Table 2 and 4. This situation agree with the results observed in other study (Cheville *et al*., 1992) in which persistence of both vaccinate strains in preescapular lymph nodes were evaluated in relation to the histological changes and induced immunological response. Other studies done in caprines vaccinated with strain RB51 showed that S19 strain is processed in an accurate way by normal immunological mechanisms before 12 weeks (Stevens *et al*., 1994b).

Measurements recorded at 180 days after vaccination with S19 strain allowed to identify differences between the vaccinated and non-vaccinated groups as it is shown in Table 5. However, these differences are observed when a larger number of reactor females are found at non-vaccinated group. In addition, shows again that also S19 strains, does not induce perdurable macroscopic changes in the preescapular lymph nodes (Cheville *et al*., 1992). Results are very similar to other research studies (Baldwin and Parent, 2002) in order to despite that the vaccinate strains are eliminated from the organism of the vaccinated animals between 3-4 weeks.

It is worthy to say that as time passes vaccine reaction decreases. However, this does not mean that the animals will be unprotected (Martinez-Herrera *et al*., 2002) because memory cells are in the organism particularly in the lymph nodes where will show up in the presence of germination centers (Cheville *et al*., 1992) and last the whole animals useful productive life (Stevens *et al*., 1997). Finally in order to explain dimensions increase from lymph nodes for non-vaccinated groups of females with RB51 or 19 strain, it is important to recall that immune system has the ability to respond immediately to any antigenic stimuli (Tizard, 2004) which means that there are factors capable to induce lymphatic adenomegalic reactions such as tuberculosis, actinomycosis and leucosis, etc. (Jackson and Cockcroft, 2002). For these reasons it can be assumed that rises in node dimensions in vaccinated and non-vaccinated animals between 90 and 180 days after vaccination are not a consequence of exposition to vaccine strains.

CONCLUSION

It is conclusive that dimension from preescapular lymph nodes in bovines vaccinated against brucellosis have a mean size of 3.7-4.8×10-11.7 cm and in the non-vaccinated one is of 3.8-4.5×10-11.5 cm. On the other hand, the available vaccinated strains in Mexico are processed in an efficient way by the lymph tissue of dual purpose bovines in the tropic of Veracruz. There is no difference neither in lymph tissue rise ($p<0.05$) vaccinated and non-vaccinated bovine nor in the kind of strain used. Both available vaccine strain doses in Mexico are safe because they do not induce inflammation in the area where it is applied and they do not threatened the physical integrity of vaccinated animals.

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