International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 4 (3): 109-116, 2009
ISSN 1811-9743

Evaluation of Vaccination with *Brucella abortus* RB51 Strain in Herds Naturally Infected with Brucellosis in Productive Systems Found in Tropical Climate

[1]A. Peniche Cardeña, [1]D. Martínez Herrera, [1]J.L. Franco Zamora,
[3]F. Barradas Piña, [1]B. Molina Sánchez, [2]E.J. Gutiérrez Ruíz,
[2]J.J. Williams, [4]F. Morales Alvarez and [4]R. Flores Castro
[1]FMVZ. Universidad Veracruzana,
[2]FMVZ. Universidad Autónoma de Yucatán,
[3]CE La Posta. INIFAP, México
[4]CENID-Microbiología Animal, INIFAP, México

Abstract: In this study, the efficacy of vaccination with *Brucella abortus* RB51 strain as a measure for bovine brucellosis control was evaluated by a clinical assay in double purpose cattle that are naturally infected under tropical conditions. A herd with eight reactors to rivanol test with an initial serum reaction of 5% was selected. Confirmation of infected herd was carried out by isolation and identification of *Brucella abortus* from reactor animals, using bacteriological procedures. Also, the milk samples were analyzed by PCR technique whereby *Brucella abortus* infection was corroborated. Vaccinated and non-vaccinated groups were formed with 88 females each. Reactors were not eliminated nor segregated from the population. During 18 months of monitoring three new cases happened in the vaccinated group and therefore the initial serum reaction rate increased from 10 to 12.5%. The rate of vaccinated group remained at 0% due to 100% of protective efficacy that RB51 strain provided to the total vaccinated population (RR = 0; C.I. 95% 0-0). The conclusion is that under extensive double purpose livestock rearing conditions tropical climate, strain RB51 is a biological product efficacious for brucellosis control in infected herds with a prevalence of 6%.

Key words: *Brucella abortus*, bovine brucellosis, RB51 vaccine, RB51 strain

INTRODUCTION

Vaccination is an indispensable practice in the control of bovine brucellosis and at the international level the RB51 and 19 strains of *Brucella abortus* (Halling and Boyle, 2002). *Brucella abortus* RB51 strain does not have the "O" polysaccharide and therefore, it does not induce the formation of antibodies against this bacterial lipopolysaccharide fraction. Thus, when animals are vaccinated with this strain it does not interfere with the routine diagnostic serology tests and thus it allows identification of vaccinated animals separating them from the infected ones; this does not happen with strain 19 (Moriyon *et al*., 2004). The use of strain RB51 was integrated into the eradication programs of the disease in Colombia, Costa Rica, Chile, United States of America, Mexico, Paraguay, Uruguay and Venezuela. Nevertheless, Argentina suspended its use due to a low protective response

Corresponding Author: D. Martínez Herrera, Ph.D. Universidad Veracruzana, México

(Samartino *et al*., 2000; Garin *et al*., 2005). In view of the above, controversies on its efficacy allow us to suppose that there are important differences in test results provided by controlled experiments and how the vaccines really work in the field, exposed to different conditions and challenges (Aparicio *et al*., 2003; Samartino, 2005). Therefore, the objective of this study was the evaluation of the RB51 strain vaccine in herds that had cattle naturally infected with brucellosis under tropical conditions.

MATERIALS AND METHODS

Study Site

This research was carried out in the El Desengaño, community in Las Choapas, Municipality of Veracruz, Mexico between August 2006 and February 2008. During the first stage a transverse epidemiological study was carried out in order to identify herds that were naturally infected with brucellosis.

Inclusion Criteria

Units dedicated to double purpose production in an extensive system in a tropical climate and without brucellosis vaccination were the ones taken into consideration. All animals six months old or more were sampled according to NOM-041-ZOO-1995 National Campaign against Brucellosis in Animals (SAGDR, 1996). The infected herd was defined as that one where there were animals, reactors to the buffered-tampon antigen test or Card Test (CT) with antigen at an 8% concentration and with at least one positive case to the precipitation action by Rivanol Test (RT).

Serology Diagnosis

Five milliliter blood sample was taken from the coccygeal vein with vacutainer without anticoagulant. Samples were transported in refrigeration to the Microbiology Laboratory of the Faculty of Veterinary Medicine and Animal Husbandry of the University of Veracruz. The serum was placed in vials identified with the sample number and preserved at -20°C until processing by the CENID Laboratory - Animal Microbiology of INIFAP in Palo Alto, D.F., by CT and RT according to NOM-041-ZOO-1995 National Campaign against Brucellosis in Animals. By RT any agglutination value equal to or above 1:25 was considered as positive (SAGDR, 1996).

Clinical Assay

Based on the inclusion criteria, for the clinical assay a herd infected with brucellosis was selected. To estimate the sample size and establish the vaccinated and non-vaccinated groups the 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 potency of 80%. Thus, the sample size was estimated at 88 animals per group (Thrusfield *et al*., 2001). Vaccinated and non-vaccinated groups were randomly selected and identified by ear tags. From the time of vaccination, both groups were evaluated quarterly by serology using CT and RT tests during 18 months in modalities of screening and confirmatory, respectively.

Vaccination

All females that had negative results to CT and RT were vaccinated subcutaneously once, in the middle third of the neck on the left side. Strain RB51 vaccine was used in

doses of 5×10^{10} Colony Forming Units (CFU) in females 6 to 12 months of age and in doses of 3×10^{8} to 3×10^{9} CFU in animals older than 12 months including gestating females (SAGDR, 1996). Vaccination of animals was carried out in the month of August 2006; at the time of experimental group establishment, 32 gestating females were integrated into the vaccinated group and 36 gestating females in the non-vaccinated group. Males were not vaccinated and animals seropositive to RT were not segregated or eliminated from the herd.

Statistical Analysis

To determine seroprevalence rates, Relative Risk (RR) and Confidence Intervals (CI) of 95% were estimated according to Thrusfield (2005). Statistical significance of observed frequencies in vaccinated and non-vaccinated groups was estimated by Chi-square and significant differences were considered when $p<0.05$ (Daniel, 1999).

Vaccination Efficacy

It was estimated by the formula (Oresntein *et al*., 1985):

$$VE = \frac{CDR - VDR}{CDR} \times 100$$

Where:
VE = Vaccination efficacy
CDR = Diseased animal rate within the control group
VDR = Diseased animal rate within the vaccinated group

Bacteria Isolation

Bacteria isolation was considered an inclusion criteria necessary to confirm infection of the herd by *Brucella abortus* and thus, be able to evaluate efficacy of strain RB51 in the presence of field strains; therefore, in each monitoring, milk samples were collected in sterile Falcon type tubes and bacteria isolation was carried out following procedure by Alton *et al*. (1988) of all animals reactors to RT. Samples were maintained in refrigeration from the time of collection until processed. From the milk fat, duplicate primary seeding was carried out in Farrell selective media and incubated in aerobiosis and micro-aerobiosis environments; media were incubated at least during one month at 37°C and checked for colony development every other day. Isolations suggestive of *Brucella* sp. by colony morphology were seeded again in Trypticasein Soy Agar (TSA) until pure cultures were obtained and identified by biochemistry tests. Also, samples were sent refrigerated to the Microbiology Department of the National School of Biological Sciences of the National Polytechnic Institute, for confirmation diagnosis by Polymerase Chain Reaction (PCR) (Matar *et al*., 1996; Hamdy and Amin, 2002).

RESULTS

Serological Diagnosis

The transverse selection study allowed the identification of a brucellosis infected herd by a serum reaction rate to RT of 5% (8/176) with agglutination reactions between 1:25 and 1:400 that were considered positive in non-vaccinated animals as established in NOM-041-ZOO-1995 National Campaign against Brucellosis in Animals (SAGDR, 1996).

Clinical Assay

In Table 1, we are found the new cases of animals that were reactors to RT during each one of the quarterly post vaccination follow-up monitoring.

In the first year of monitoring three new infections cases happened in the non-vaccinated animals (Table 1). During all the research the rate of serum reaction of the non-vaccinated group (CDR) increased from the initial 10 to 12.5% in 18 months, while in the vaccinated group (VDR) it was 0%. Dissemination of the disease in animals of the non-vaccinated group propitiated an increase of the initial serum reaction rate of 5% to an accumulated serum reaction rate of 6% in the herd during the study period (Fig. 1).

When estimating frequencies observed in the vaccinated and non-vaccinated groups a year and half after vaccination, significant differences were found between them ($p<0.05$); nevertheless no association was found between groups and serum conversion (RR = 0; C.I. 95% 0-0) and this indicates that strain RB51 vaccine protected the total amount of susceptible population. It must be underlined that none of the 32 gestating females of the vaccinated group aborted as a consequence of the biological product application nor did they develop the disease.

Vaccination Efficacy

Since, no serum positive or reactor animal was detected in the vaccinated group during the 18 months of the research, the strain RB51 vaccine of *Brucella abortus* had a protective efficacy of 100%.

Bacteria Isolation

In cultures carried out in micro-aerobiosis of the 26 milk samples, *Brucella abortus* colonies showed up in 38% of the cases (10/26); for this, 10 reactor animals were monitored and the quarterly isolations that were obtained came from 60% of these (6/10) as can be seen in Table 2.

Table 1: New cases seropositive to rivanol test identified during quarterly serology monitoring in the non-vaccinated group

Ear tag	Rivanol reaction	Post vaccination quarter identification
555	1:50	First
391	1:50	Third
455	1:50	Fourth

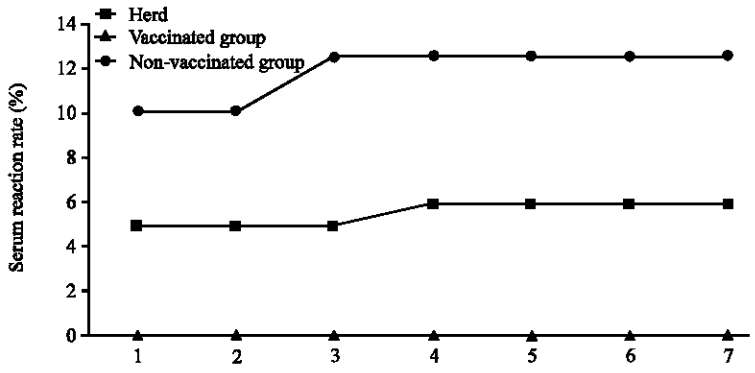

Fig. 1: Serum reaction rates in the vaccinated and non-vaccinated groups and the herd during quarterly follow-up monitoring

Table 2: Isolation of *Brucella abortus* from milk of animals reactors to rivanol test during the study

Month/year													
Aug./06		Nov./06		Feb./07		May./07		Aug./07		Nov./07		Feb./08	
Num.	I	Num.	I	Num.	I	Num.	I	Num.	I	Num.	I	Num.	I
457	+	375	+	389	-	375	+	375	+	375	+	375	-
507	-	439	-	439	-	389	-	391	+	391	-	389	-
517	+	517	+	465	-	465	+	439	-	465	-	455	-
						555	-	517	-			465	-
								555	-			555	+

Num.: Identification, I: Isolation, +: Positive, −: Negative

Isolations that were obtained were confirmed as *Brucella abortus* through PCR studies carried out from the collected milk by the use of primers that amplify membrane protein OMP´s 31 kDa.

DISCUSSION

Serology Diagnosis

Rivanol Test (RT) test has a relative sensitivity between 86 to 97% and therefore it is not recommended for eradication program final stages (Dájer-Abimerhi *et al*., 1998); nevertheless, due to its high specificity (100%) it identifies IgG antibodies derived from a strong antigenic stimulus and therefore its presence implies an active infection, or chronic infection making it useful as a confirming test in control programs or early stages of eradication campaigns (Dájer-Abimerhi *et al*., 1995; Díaz *et al*., 2001). The eight positive cases to RT that were identified during the transverse study were females older than three years old, situation that coincides with what Nicoletti (2005) mentions, referring to the fact that the infection affects bovines of all ages but persists more frequently in sexually developed animals.

Clinical Assay

In 18 months of research a total of three new infection cases were identified in the non-vaccinated group, in this sense, Rentería *et al*. (2003) and Nicoletti (2005) underline that the degree of crowding and animal population density are factors that favor the transmission of the disease since thus there is a higher probability that susceptible animals be exposed to the infection; nevertheless, Magaña-Monforte *et al*. (2006) indicated that neither crowding nor population density are factors that characterize the extensive cattle production system and therefore since that does not influence the population, contact between diseased and susceptible animals is not facilitated. This in turn reduces the risk of infection; this together with a low disease incidence observed during the study could explain the reduced number of new cases identified during the first year in the animals of the non vaccinated group.

Immunization with strain RB51 reduces susceptibility to the infection by providing immune protection from three to four weeks after its application and slowly reduces the level of exposure to the infection since the number of infected animals with brucellosis does not increase in the herd (Casas, 2003); this explains why the rate of serum reactors in the vaccinated group (VDR) was 0% and of the non-vaccinated group (CDR) was increased from the initial 10% to 12.5% during the study period. Different from what Van Metre *et al*. (1999) found in his study, reporting the infection of a gestating vaccinated female, during this study no gestating female of the vaccinated group aborted and neither did it get infected with brucellosis. In their respective studies, Edmonds *et al*. (1999) and Olsen (2000) come to the conclusion that vaccination with RB51 strain does not cause reproductive problems or abortion when applied to sexually mature or gestating females; these statements, coincide with observations of this study, of gestating females of the vaccinated group. Vaccination

of females even during the last third of gestation with strain RB51 with doses of 3×10^9 CFU as used in this study, is considered by Uza *et al.* (2000) as safe, since there is no diagnostic interference and it does not cause abortion.

Vaccination Efficacy

Leal *et al.* (2005) mentioned that in endemic zones, strain RB51 protects up to 94% of the vaccinated herd when challenged by field virulent strains; thus, the 100% protective efficacy provided by strain RB51 to animals of the vaccinated group, is above the rate reported by these authors. The obtained result of vaccine efficacy coincides with that which was expressed by Lord *et al.* (1998) and Ramírez *et al.* (2002), who indicated that this strain does not induce serum conversion in vaccinated females and protects 100% of the susceptible cattle when it is used to control brucellosis in low prevalence herds.

Bacteria Isolation

As it is seen in Table 2 consecutive, alternate or only isolations were obtained from the milk samples that came from reactor animals and this may be due among other factors to the presence of the bacteria in this product. Osorio (2004) mentioned that the bacteriological procedures is not always successful due to the intermittency in the elimination of the bacteria in milk; together with that, Rentería *et al.* (2005) indicated that a large amount of bacteria are needed in the sample or that the sample must be collected when *Brucella* sp. is being excreted.

Rodríguez *et al.* (2005) indicated that approximately 50% of infected cows eliminate *Brucella* spp. in milk during several weeks or months after aborting or calving; notwithstanding the above, in this study 60% of the infected monitored cows eliminated the bacteria during the six quarters, situation that favored the isolation of the same in animals in production as well as in the dry period, in which, as indicated by these researchers, elimination is reinforced.

López *et al.* (1992) reported that in milk and vaginal secretions approximately 10 bacteria/gram are eliminated even in the cases where no symptoms are observed, as was the clinical status presented by all reactor animals during this study. Milk of affected animals facilitates contamination of the environment and favors the dissemination and transmission of the disease to the susceptible population, especially in those farms where hygiene is deficient and there is the custom of throwing the first spurt of milk on the floor prior to milking (despunte in Spanish) (Rodríguez *et al.*, 2005); this is a procedure that is carried out in this production unit during milking.

According to what has been mentioned by Dájer-Abimerhi *et al.* (1998) and Díaz *et al.* (2001), isolation and identification of the bacteria obtained during 5 quarters of field monitoring together with the presence of new cases, allowed the confirmation of infected herd.

Moreno *et al.* (2002) and Rentería *et al.* (2003) consider that the reactor animals within the population are a risk factor that favors the transmission and permanence of the disease in the herd. The presence of a circulating field strain of *Brucella abortus* that comes from the reactor cattle and that has not been eliminated, propitiated that vaccinated and not-vaccinated animals had at all times a constant natural challenge, that allows the evaluation of the protective efficacy of strain RB51.

Results of this study allows us to conclude that under extensive double purpose cattle production system in tropical climate, strain RB51 is an efficacious biological product for the control of bovine brucellosis in naturally infected herds with a prevalence of 6%.

ACKNOWLEDGMENTS

This study is part of the requirements that the first author must cover to obtain the degree of Doctor in Agriculture and Livestock Sciences granted by the Autonomous University of Yucatan who carry out an economic support with a CONACYT scholarship holder. 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

Alton, G.G., L.M. Jones, R.D. Angus and J.M. Verger, 1988. Techniques for the Brucellosis Laboratory. 1st Edn., Institute Nationale de le Rech, France, Paris, pp: 174.

Aparicio, B.A., E.D. Aparicio, L.H. Andrade, R.P. González, E.A. Silva and F.S. Güemes, 2003. Evaluación serológica y bacteriológica de un hato bovino con brucelosis y revacunado con dosis reducida de Brucella abortus cepa 19. Tec. Pecu. Mex., 41: 129-140.

Casas, O.R., 2003. Informe sobre vacunas y vacunación contra brucelosis bovina. Vet. Montevideo, 38: 31-41.

Dájer-Abimerhi, A., E.J. Gutiérrez-Ruiz, D Zapata-Villalobos, N. Honhold and S.L. Villegas-Pérez, 1995. Comparación de cinco pruebas serológicas para la detección de anticuerpos contra Brucella abortus y reporte preliminar del porcentaje de reactores positivos en hatos bovinos en Yucatán, México. Rev. Biomed., 6: 84-90.

Dájer-Abimerhi, A.F., E.J. Gutiérrez and D. Zapata, 1998. Uso de las pruebas de ensayo inmunoabsorbente ligado a enzimas y aglutinación con rivanol para el diagnóstico de brucelosis bovina en Yucatán, México. Vet. México, 29: 167-171.

Daniel, W.W., 1999. Biostastistics: A Foundation for Analysis in the Health Sciences. 7th Edn., Wiley, New York, pp: 907.

Díaz, A.E., H.M. Leal and C.A. Cantu, 2001. Bovine Brucellosis. In: Animal Brucellosis Diagnostic, Di–az, E., L. Hernendez, G. Valero and B. Arellano (Eds.). INIFAP, Mexico, D.F., pp: 136-139.

Edmonds, M.D., G. Schurig, L. Samartino, G. Hoyt, V. Walker, D. Hagius and H. Elzer, 1999. Biosafety of *Brucella abortus* strain RB51 for vaccination of mature bulls and pregnant heifers. Am. J. Vet. Res., 60: 722-725.

Garin, A., A.D. Gil, M. Silva, O. Caponi, L. Chans and E. Vitale, 2005. Brucellosis eradication program in Uruguay. Proccedings of the 58th Internacional Research Conference, Oct. 15-19, Merida (Yucatan) Mexico, pp: 96-96.

Halling, S.M. and M. Boyle, 2002. Foreword. Vet. Microbiol., 90: 1-3.

Hamdy, M.E. and M. Amin, 2002. Detection of *Brucella* species in the milk of infected cattle, sheep, goats and camels by PCR. Vet. J., 163: 299-305.

Leal, H.M., E. Díaz, R. Pérez, H. Andrade, B. Arellano, E. Alfonseca and F. Suárez, 2005. Protection of *Brucella abortus* RB51 revaccinated cows, introduced in a herd with active brucellosis, with presence of atypical humoral response. Comp. Immunol. Microbiol. Infect. Dis., 28: 63-70.

Lord, V.R., G. Schurig, W. Cherwonogrodzky, J. Marcano and E. Melendez, 1998. Field study of vaccination of cattle with *Brucella abortus* strains RB51 and 19 under high and low disease prevalence. Am. J. Vet. Res., 59: 1016-1020.

López, M.A., R. Migranas, A. Pérez, C. Magos, B. Salvatierra, R. Tapia, L. Valdespino and J. Sepúlveda, 1992. Seroepidemiología de la brucelosis en México. Rev. Salud Publica de México, 34: 230-240.

Magaña-Monforte, J.G., G. Rios-Arjona and J.C. Martínez-González, 2006. Dual purpose cattle production systems and the challenges of the tropics of Mexico. Arch. Latinoam. Prod. Anim., 14: 105-114.

Matar, G.M., I.A. Khneisser and A.M. Abdelnoor, 1996. Rapid laboratory confirmation of human brucellosis by PCR analysis of a target sequence on the 31-kilodalton *Brucella* antigen DNA. J. Clin. Microbiol., 34: 477-478.

Moreno, R.J.F., T. Renteri–a, R. Searcy and F. Montano, 2002. Seroprevalencia y factores de riesgo asociados a brucelosis en hatos lecheros de Tijuana, Baja California. Tec. Pec. Mexico, 40: 243-249.

Moriyon, I., J. Grillo, D. Monreal, D. Gonzalez and C. Marin *et al*., 2004. Rough vaccines in animal brucellosis: structural and genetic basis and present status. Vet. Res., 35: 1-38.

Nicoletti, P., 2005. Epidemiology in Brucellosis. Proccedings of the 58th Internacional Research Conference, October 15-19, Merida (Yucatan) Mexico, pp: 1-6.

Olsen, S.C., 2000. Responses of adult cattle to vaccination with a reduced dose of *Brucella abortus* strain RB51. Res. Vet. Sci., 69: 135-140.

Oresntein, W.A., H. Bernier, J. Dondero, R. Hinman, S. Marks, J. Bart and B. Sirotkin, 1985. Field evaluation of vaccine efficacy. Bull. World Health Organ., 63: 1055-1068.

Osorio, M.F.J., 2004. Brucelosis y estrategias para su control. I Simposium Internacional de Enfermedades Emergentes y Re-emergentes, Feb. 26-27, Barranquilla, Colombia, pp: 466-467.

Ramírez, M., S. Ernst, F. Elvinger, A. Rivera and C. Rosenfeld, 2002. Respuesta serológica y tiempo de saneamiento en rebanos bovinos con brucelosis vacunados con cepa 19 o cepa RB51: X^{a}. Region, Chile. Arch. Med. Vet., 34: 213-220.

Rentería, E.T.B., K. Nielsen, A.F.L. Navarro, M.F.M. Gómez and J.F.M. Rosales, 2003. Evaluacion de un programa de control de la brucelosis bovina en hatos lecheros de Baja California. Tec. Pecu. Mexico, 41: 275-282.

Rentería, E.T.B., H. Organes de los Santos, F. Licea, E. Medina and K. Nielsen *et al*., 2005. Evaluation of the polymerase chain reaction test (PCR), for the diagnosis of bovine brucellosis. Tec. Pecu. Mexico, 43: 117-126.

Rodríguez, V.Y., S.W. Ramírez, S.G. Antúnez, F. Pérez, P.Y. Ramírez and A. Igarza, 2005. Brucelosis bovina aspectos historicos y epidemiologicos. Revista Elec. de Vet. Redvet. Vol. 9. http://www.veterinaria.org/revistas/redvet/n090905.html.

SAGDR, 1996. Norma Oficial Mexicana, NOM-041-ZOO-1995. Campaña Nacional Contra la Brucelosis en los Animals. Diario Oficial de la Federación. Mexico, D.F.

Samartino, L.E., M. Fort, R. Gregoret and G. Schurig, 2000. Use of *Brucella abortus* vaccine strain RB51 in pregnant cows after calfhood vaccination with strain 19 in Argentina. Prev. Vet. Med., 45: 193-199.

Samartino, L.E., 2005. Brucellosis vaccines. Proccedings of the 58th Internacional Research Conference, October 15-19, Merida (Yucatan) Mexico, pp: 31-41.

Thrusfield., M., C. Ortega, I. de Blas, P. Noordhuizen and K. Frankena, 2001. Win Episcope 2.0: Improved epidemiological software for veterinary medicine. Vet. Record, 148: 567-572.

Thrusfield, M., 2005. Veterinary Epidemiology. 3rd Edn., Blackwell Publishing, Incorporated, Ames, Iowa EUA.

Uza, F.A., L. Samartino, G. Schurig, A. Carrasco, K. Nielsen, F. Cabrera and R. Taddeo, 2000. Effect of vaccination with *Brucella abortus* strain RB51 on heifers and pregnant cattle. Vet. Res. Commun., 24: 143-151.

Van Metre, D.C., A. Kennedy, C. Olsen, R. Hansen and R. Ewalt, 1999. Brucellosis induced by RB51 vaccine in a pregnant heifer. J. Am. Vet. Med. Assoc., 215: 1491-1493.