International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 4 (4): 142-151, 2009
ISSN 1811-9743

Evaluation of Vaccination with *Brucella abortus* S19 Vaccine in Cattle Naturally Infected with Brucellosis in Productive Systems Found in the Mexican Tropic

[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, Mexico
[4]CENID-Microbiología Animal. INIFAP, Mexico

Abstract: Efficacy of vaccination with *Brucella abortus* S19 vaccine as control measure against bovine brucellosis has been controversial; therefore, it is necessary to know the efficacy of this vaccine under different field conditions. In this study, a clinical assay was performed to establish the efficacy of this vaccine on double purpose cattle. Two groups of one hundred animals each were formed. Infected cattle were not eliminated or segregated. One herd was identified as infected, with four animal reactors to Card Test (CT) and confirmed by Rivanol Test (RT) with a serum reaction rate of 1.2%. Confirmation of infected herd was carried out by isolation and identification of *Brucella abortus* colonies and PCR on milk samples from RT reactor animals. In 18 months, the number of infected animals increased to eight females, seven within the non-vaccinated group and one in the vaccinated group for a serum reaction rate in the non-vaccinated group of 5.8% and the vaccinated one of 0.8%. Thus, in this period the accumulated serum reaction rate for both groups was increased from 1.2 to 3%. Vaccination efficacy of strain S19 was 86% and the risk of getting the disease in these animals was very low (RR = 0.112; $I.C._{95\%}$ 0.014-0.887). It is concluded that strain S19 vaccine is efficacious in the control of brucellosis in herds with a 3% prevalence of the disease; yet, before its use, interference of diagnosis problems that are produced should be assessed to properly evaluate economics and vaccination efficiency.

Key words: *Brucella abortus*, S19 vaccine, vaccine efficacy, cattle

INTRODUCTION

In Mexico, bovine brucellosis control has been carried out since 1951 using the S19 strain of *Brucella abortus*, nevertheless, when in 1997 the RB51 strain appeared, its use tapered down significantly even though today there is still a large amount of cows that have been vaccinated with RB51 vaccine (Bustamante *et al*., 2000).

A disadvantage of the S19 strain is that it induces permanence of agglutinins in serum and milk, propitiating interference with diagnosis since there is no discrimination of cattle

Corresponding Author: D. Martínez Herrera, Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana, Circunvalación s/n esq, Yañez, Col., Unidad Veracruzana, Veracruz, Ver., Mexico, C.P. 91710, Mexico
Tel/Fax: 052 229 9342075

infected with field strains and that vaccinated with S19 vaccine (Saldarriaga and Rugeles, 2002; Castro *et al*., 2005). To solve this problem, diagnostic tests should be used that are more specific, such as Radial Immunodiffusion (RID) that has proven in strain S19 vaccinated adult cattle that it is more sensitive and specific than the complement fixation test, since it has the capacity to identify individuals that do not have the disease by the determination of the Native Hapten (NH) that is an important antigenic polysaccharide in nature, component of *Brucella abortus* (Bustamante *et al*., 2000; González *et al*., 2006).

Notwithstanding this diagnostic disadvantage, Aparicio *et al*. (2003) and Samartino (2005) stated that contribution and benefits in the use of S19 strain in Campaign programs to control ruminant brucellosis outweigh such disadvantage. Also, they reiterate that in view of the controversy that preventive failure reported in immunized herds has caused it is necessary that its use be reevaluated to establish their efficacy in the control of the disease when exposed to different field conditions and challenges. Therefore, the objective of this study was to evaluate *Brucella abortus* S19 vaccine in cattle naturally infected with brucellosis under tropical conditions.

MATERIALS AND METHODS

Study Site

Research was carried out in the El Desengaño community, Municipality of Las Choapas, Veracruz, Mexico between August 2006 and February 2008 (18 months); in the first stage, a transverse epidemiological study was carried out to identify herds naturally infected with brucellosis (Toma *et al*., 1999).

Inclusion Criteria

Units that in tropical climate had a double purpose, grazing production system, which had not vaccinated animals against brucellosis, were selected. Samples were taken from animals six months or older. Infected herds were those that had reactors to the tampon-antigen test or card test with antigen at 8% concentration (CT) and at least one positive case to Rivanol Test (RT).

Serological Diagnosis

Five milliliter of blood were obtained from the coccygeal vein by vacutainer without anticoagulant. Samples were transported in refrigeration to the Microbiology Laboratory of the Veterinary Medicine and Animal Husbandry Faculty of the University of Veracruz. Sera were then placed in vials identified with sample number and preserved at -20°C until they were processed by the Animal Microbiology-CENID Laboratory of INIFAP in Palo Alto, D.F., by CT and RT according to NOM-041-ZOO-1995 National Campaign against Brucellosis in Animals. Positive animals were those that were reactors to CT, confirmed by RT, when there was titer of 1:25 or above in non-vaccinated cattle or those that had a titer of 1:50 or above in vaccinated cattle (SAGDR, 1996).

Clinical Assay

Based on the inclusion criteria of the clinical assay, a herd infected with brucellosis was selected. To estimate the sample size from the vaccinated and non-vaccinated groups the Win Episcope 2.0 program was used under the modality of finding the difference between proportions by estimating the expected proportion of 6% of animals positive to brucellosis in the vaccinated population and 20% of animals positive to brucellosis in the non-vaccinated population, with a confidence level of 95% and an 80% power. Thus the

estimated sample size was 88 animals per group, but the results were improved with 10% more animals for safety, foreseeing mortality, leaving 100 females per group (Thrusfield *et al*., 2001). Vaccinated and non-vaccinated groups were identified by ear tags with pair and impair numbers respectively; from the time of vaccination, both groups were evaluated quarterly by CT and RT serology for eighteen months as screening and confirmation tests, respectively (SAGDR, 1996). After twelve months of having applied the vaccine, all sera of the vaccinated animals group that were positive to RT were analyzed by RID in the Animal Microbiology-CENID Laboratory of INIFAP to confirm seropositivity by field strains infection (González *et al*., 2006).

Vaccination

All females that came out negative to CT and RT in the selection study were vaccinated subcutaneously one only time in the middle third on the left side of the neck. Strain S19 of *Brucella abortus* was applied in classic doses (5×10^{10} CFU) in three to six months old females and in reduced doses (3×10^{8} to 3×10^{9} CFU) in females older than six months old, including gestating ones (SAGDR, 1996). Vaccination of animals was carried out in the month of August 2006; at the time the experimental groups were being formed, 41 gestating females were integrated into the vaccinated group and 59 gestating females in the non-vaccinated group. Males were not vaccinated and seropositive animals to RT were not segregated nor eliminated from the herd.

Statistical Analysis

Determination of seroprevalence rates, Relative Risk (RR) and Confidence Intervals (CI) at 95% were estimated following Thrusfield (2005). Statistical significance of observed frequencies in vaccinated and non-vaccinated groups was estimated by chi-square and differences were considered significant if $p<0.05$ (Daniel, 1999).

Vaccination Efficacy

Vaccination efficacy was estimated by the following formula:

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

Where:
VE = Vaccination efficacy
CDR = Diseased animal rate within the control group
VDR = Diseased animal rate within the vaccinated group (Orenstein *et al*., 1985)

Bacterial Isolation

Bacterial 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 S19 in the presence of field strains; therefore, in each monitoring milk samples were collected in sterile Falcon type tubes and bacterial 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 Trypticase Soy Agar (TSA) until pure culture was 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 simple 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 1.2% (4/340) 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 the first six months of quarterly monitoring, three seropositive animals to RT were identified in the non-vaccinated group; 75% of them (2/3) were detected in the second quarter. Thus the number of animals with brucellosis in the non-vaccinated group increased from four to seven; of these six were adult females (87.5%) and the other a female one and half years old (12.5%).

In the vaccinated group a quarterly serum reaction rate to RT fluctuated between 5 and 10%, situation that implied the presence of adult reactors during the 18 months following vaccination. Nevertheless, one year after the vaccine was applied, in this group six sera were identified with positive titers to RT; to confirm if the antibodies present corresponded to a natural infection the sera were processed by RID test. Of the six sera that were analyzed, only one resulted positive so the corresponding female was diagnosed as naturally infected and the rest were identified as false positive reactors (Table 1).

During the 18 month long monitoring a total of four new infection cases were detected in the population: one in the vaccinated group and three in the non-vaccinated group, therefore the number of animals with brucellosis in the herd increased from four to eight. The presence of seropositive animals in the experimental groups propitiated an accumulated serum reaction in the vaccinated group (VDR) of 0.8% and in the non vaccinated group (CDR), the rate increased from the initial 3.3 to 5.8%. Disease dissemination in the herd favored the increase of initial serum reaction rate of 1.2% to an accumulated serum reaction rate of 3% during the study period (Fig. 1).

When estimating observed frequencies in vaccinated and non-vaccinated groups a year and a half after vaccination, significant differences were observed between them ($p<0.05$). Results that were obtained indicated that S19 strain *Brucella abortus* vaccine did not impede infection in the total susceptible vaccinated population; nevertheless the risk of infection of vaccinated animals and getting sick was very low, due to the protection effect

Table 1: Animals seropositive to RT and RID identified during the monitoring of the vaccinated group a year after vaccination

Ear tag	RT	RID
56	1:200	-
120	1:50	-
124	1:50	-
148	1:400	-
166	1:50	-
618	1:100	+

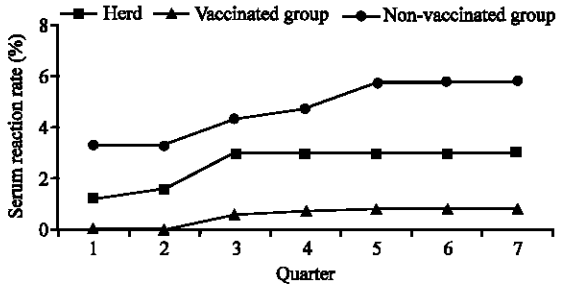

Fig. 1: Serum reaction rates of the herd and the vaccinated and non-vaccinated groups during quarterly monitoring follow-up

Table 2: Isolation of *Brucella abortus* in milk obtained from 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
137	-	137	+	137	-	137	+	137	+	57	-	57	+
173	-	173	-	173	-	139	-	173	+	139	-	139	+
181	+	181	-	181	-	181	+	181	-	618	+	618	-

Num.: Ear tag number identification, I: *Brucella abortus* isolated

of the vaccine (RR = 0.112; $IC_{95\%}$ 0.14-0.887). It must be underlined that none of the 41 gestating females of the vaccinated group aborted as a consequence of vaccine application.

Vaccine Efficacy

Since, only one female of the vaccinated group got infected during the six research quarters, strain S19 of *Brucella abortus* vaccine had a protective efficacy of 86%.

Bacterial Isolation

Brucella abortus colonies developed in 43% of the cases (9/21) of the 21 milk samples cultured in micro-aerobiosis; isolations that were obtained came from 100% (6/6) of sampled reactor animals. It is important to underline that isolation of the bacteria in milk of the vaccinated female reactor to RID, was also obtained during the monitoring that was carried out three months after having been diagnosed with brucellosis (Table 2).

Isolations were confirmed as *Brucella abortus* through PCR carried out in collected milk using primers that amplify OMP´s 31 kDa membrane protein.

DISCUSSION

Serological Diagnosis

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 program or early stages of eradication campaigns (Dájer-Abimerhi *et al*., 1995; Díaz *et al*., 2001). The four 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

After the second sampling, subsequent monitoring did not identify new infections probably due to that which was mentioned by Casas (2003), who stated that vaccination of adult females induces protection against brucellosis and the animals obtain immunity in three to four weeks after the application of the biological product, accelerating the control process of the disease.

NOM-041-ZOO-1995 National Campaign against Brucellosis in Animals, establishes that reduced doses strain S19 vaccinated animals, may be tested with official diagnostic tests only ten months after the application of the vaccine, with the purpose of detecting serum positive titers in the animals by RT test and then go on to confirmation if the positive sera correspond to infected animals or serum converting animals by more specific laboratory tests (SAGDR, 1996) following the provisions of this standard, during the fourth quarterly sampling six adult females of the vaccinated group were found to be reactors and of these only one resulted positive to RID and therefore was diagnosed as infected, three months later when the isolation of the bacteria in a sample of milk was achieved, the diagnosis of infection was ratified.

Stevens *et al*. (1994) and Ramirez *et al*. (2002) mention that the persistence of antibody titers against the O chain of the lipopolysaccharide (LPS) of the bacteria is increased when adult animals are vaccinated with strain S19; this explains the presence of animals with serum reaction to RT in the vaccinated group during the 18 months after the application of the vaccine. On the other hand, Bustamante *et al*. (2000) reported that the RT test may identify the persistence of these antibodies up to 20 months after vaccination, therefore it may not be considered as a trustworthy technique to separate strain S19 vaccinated animals from infected animals; indicating also that in view of this immunological process, reactor cattle should be recognized as serum converter or false positive until it is confirmed as an infected animal by additional diagnostic tests that have greater specificity such as RID proposed by Santiago *et al*. (1997).

Also, there is evidence provided by Cheville *et al*. (1992), Stevens *et al*. (1995) and Molina *et al*. (2004) in animals vaccinated against brucellosis, that when they are exposed to field strains of *Brucella* sp., they serum convert even though they do not get sick; nevertheless, this new challenge could be considered as secondary exposure and therefore, antibody titers tend to be higher and more persistent (Tizard, 2004). This implies that tests such as CT and RT are not efficacious enough to discriminate between infected and vaccinated animals and therefore other diagnostic tools are needed such as molecular tests, competitive ELISA or RID, to get to know the health status of the animals, situation that happens frequently in naturally infected herds (Bustamante *et al*., 2000; Santiago *et al*., 1997).

In a prolonged exposure of the bacteria to the immune system as it happens in a field infection, antibodies against NH are produced; nevertheless, these are not synthesized in a temporary exposure to the bacteria as is the case of vaccination with S19 strain. RID test identifies these antibodies and differentiates infected from vaccinated animals becoming then an important diagnostic tool that permits certainty in the decision to eliminate a serum reactor vaccinated animal from a herd (Bustamante *et al*., 2000).

Brucellosis is a disease characterized by the production of abortion after the second gestation trimester, with frequent posterior consequences such as placenta retention, metritis and endometritis (Osorio, 2004); nevertheless, in this study, no abortions occurred in any of the serum positive females of the non-vaccinated group that were beyond the second third of gestation. This observation could be explained based on statements by Rodríguez *et al.* (2005), who estimated that in a herd, 65% of infected females abort and of these 80% do so only one time during their productive life; the rest carry their gestation full term, as it happened in animals of this group. This leads us to believe that infection in reactor animals could have been chronic and therefore abortion could have already occurred before the observation period was begun. Females with advanced gestation in the vaccinated group, also did not present abortions. Nicoletti (1976) mentioned that the application of S19 strain vaccine in females at the final stages of gestation does not cause abortion in more than 1% of the animals; this statement coincides with observations made during this field study.

Vaccine Efficacy

Experiences with reduced doses have shown that S19 vaccine induces an effective protection in bovines. Nicoletti (1976), Casas (2003) and Samartino (2005) indicated that cattle immunization with this strain favors a 65 to 75% protection of vaccinated animals; in the rest of the population, a relative defense is stimulated since even though the bovine is vaccinated, it can become infected as it happened during this study with one of the females of the vaccinated group. Notwithstanding this fact, 86% protective efficacy of strain S19 applied as single doses, obtained during this field research, is higher than what was achieved by the researchers of reference.

Bacterial Isolation

As it is observed in Table 2, isolations that were obtained corresponded to six RT reactor animals that were sampled. This result is higher in frequency than what was published by Rodríguez *et al.* (2005), who mentioned that approximately half of the infected cows after aborting or calving eliminate *Brucella abortus* in milk during several weeks or months In this study, in two of the six reactor females that were monitored there was a positive isolation at least in two occasions; which in turn corroborates the presence and elimination of the bacteria in milk during several months, situation that favors transmission of the disease in a herd but above it all it also implies a high risk for public health.

In Table 2, it can also be seen that from all post-vaccination sampling, at least one positive isolation was obtained except during the second monitoring. Consecutive, alternate or only isolations from milk samples coming from animal reactors to RT may be due to the interrupted presence of the bacteria in milk. In relation to this, Osorio (2004) mentioned that the bacteriological procedure is not always successful due to the intermittence of *Brucella* sp., elimination in milk; likewise, Rentería *et al.* (2005) emphasized the fact that a large amount of them are needed in the sample or that it is collected at the time of excretion. Also, it is important that the highest elimination of bacteria in milk has been recorded after calving and the lowest during lactation peak (Rodríguez *et al.*, 2005).

According to what has been mentioned by Dájer-Abimerhi *et al.* (1998) and Díaz *et al.* (2001), bacteria isolation and identification together with the presence of new cases during the investigation, gave way to a definite diagnosis of an infected herd.

Existence of reactor animals within the population constitutes a risk factor that favors disease transmission and permanence in the herd (Moreno *et al.*, 2002; Rentería *et al.*, 2003). Presence of *Brucella abortus* as a circulating field strain coming from reactor cattle that

were not eliminated, propitiated that vaccinated and non-vaccinated animals had at all times a natural, constant challenge permitting the evaluation of the protective efficacy of strain S19.

CONCLUSION

Results of this investigation allow the conclusion that strain S19 of *Brucella abortus* is a biological product that may be efficacious in the control of bovine brucellosis in double purpose cattle that have a disease prevalence of 3%; yet, before its application, diagnostic interference problems that characterize it should be taken into consideration in order to evaluate from the economic point of view, vaccination efficiency.

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. Vet. Res. Commun., 13: 420-420.

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.

Bustamante, S.J., H.F. Salazar, E. Díaz, C. Manzano, R. Pérez and L. Hernández, 2000. Estudio bacteriológico y serológico de brucelosis en vacas revacunadas con dosis reducida de cepa 19 de *Brucella abortus*. Tec. Pec. Mex., 38: 35-42.

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

Castro, H.A., S.R. González and M.I. Prat, 2005. Brucelosis una revisión práctica. Acta Bioquím Clin. Latinoamericana, 39: 203-216.

Cheville, N.F., A.E. Jensen, S.M. Halling, F.M. Tatum and D.C. Morfitt *et al*., 1992. Bacterial survival lymph node changes, and immunologic responses of cattle vaccinated with standard and mutant strains of *Brucella abortus*. Am. J. Vet. Res., 53: 1881-1888.

Daniel, W.W., 1999. Bioestadística. Base para el análisis de las ciencias de la salud. Uteha Noriega, México, D.F.

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.

Dájer-Abimerhi, A.F., R.E.J. Gutiérrez, V.D. Zapata, N. Honhold and P.S.L. Villegas, 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.

Díaz, A.E., H.M. Leal and C.A. Cantú, 2001. Brucelosis Bovina. In: Diagnóstico de Brucelosis Animal, Díaz, E., L. Hernández, G. Valero and B. Arellano (Eds.). INIFAP, México, D.F., pp: 136-139.

González, M.E., A.L. Hernández and A.E. Díaz, 2006. Prueba de inmunodifusión radial con hapteno nativo para diferenciar bovinos con revacunaciones repetidas con la cepa 19 de *Brucella abortus*. Tec. Pecu. Méx. 44: 269-276.

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

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.

Molina, S.B., H .D.I. Martínez, G.M.A. Abeledo, L.A. Moreno, C.M.A. Rodríguez, R.E.L. Rivera and B.R. Bautista, 2004. Duración de la protección conferida en cabras vacunadas con cepa RB51 DE *Brucella abortus*. Avances en la Investigación Agrícola, Pecuaria, Forestal y Acuícola en el Trópico Mexicano, Libro Científico No. 1. INIFAP. México, D.F., pp: 443.

Moreno, R.J.F., E.T.B. Rentería, R.S. Bernal and G.M.F. Montano, 2002. Seroprevalence and risk factors associated to bovine brucellosis of dairy herds at Tijuana, Baja California. Tec. Pec. Mexico, 40: 243-249.

Nicoletti, P., 1976. The effects of adult cattle vaccination with strain 19 on the incidence of Brucellosis in dairy herds in Florida and Puerto Rico. Proc. Annu. Meet. US Anim. Health Assoc., 83: 75-80.

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

Orenstein, W.A., R.H. Bernier, T.J. Dondero, A.R. Hinman, J.S. Marks, K.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. Proceedings of the 1 Simposium Internacional de Enfermedades Emergentes y Re-emergentes, Feb. 26-27, Barranquilla, Colombia, pp: 466-467.

Ramirez, 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., H. Organes de los Santos, N.A.F. Licea, B.G.E. Medina, K. Nielsen, G.M.F. Montano, R.J.F. Moreno and M.L.C. Pujol, 2005. Evaluación de la prueba reacción en cadena de la polimerasa (PCR) a partir de muestras de leche y cultivos puros en el diagnóstico de la brucelosis bovina. Tec. Pecu. Méx. 43: 117-126.

Rentería, E.T.B., K. Nielsen, N.A.F. Licea, G.M.F. Montano and R.J.F. Moreno, 2003. Evaluacion de un programa de control de la brucelosis bovina en hatos lecheros de Baja California. Tec. Pecu. Mexico, 41: 275-282.

Rodríguez, V.Y., S.W. Ramírez, S.G. Antúnez, B.F. Pérez, P.Y. Ramírez and P.A. Igarza, 2005. Brucelosis bovina, aspectos históricos y epidemiológicos. Revista Electrónica de Veterinaria REDVET, 6: 1-9.

SAGDR, 1996. Secretaría de Agricultura, Ganadería y Desarrollo Rural. Norma Oficial Mexicana NOM-041-ZOO-1995 Campaña Nacional Contra la Brucelosis en Animales. México, D.F., pp: 43-66.

Saldarriaga, O.A. and M.T. Rugeles, 2002. Inmunobiología de la infección por *Brucella* spp. fundamentos para una estrategia vacunal. Rev. Col. Cienc. Pec., 15: 188-197.

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

Santiago, S.B., M.E. González, M.L.A. Navarro, A.L. Hernández and A.E. Díaz, 1997. Utilización de la prueba de inmunodifusión radial para diferenciar bovinos con revacunaciones repetidas de *Brucella abortus* cepa 19. Proceedings of the 33th Reunión Nacional de Investigación Pecuaria, Nov. 3-8, Veracruz (Veracruz) México, pp: 256-256.

Stevens, M.G., S.G. Hennager, S.C. Olsen and N.F. Cheville, 1994. Serologic responses in diagnostic tests for brucellosis in cattle vaccinated with *Brucella abortus* 19 or RB51. J. Clin. Microbiol., 32: 1065-1066.

Stevens, M.G., S.C. Olsen and N.F. Cheville, 1995. Comparative analysis of immune responses in cattle vaccinated with *Brucella abortus* strain 19 or strain RB51. Vet. Immunol. Immunopathol., 44: 223-235.

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

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

Tizard, I.R., 2004. Veterinary Immunology an Introduction. Saunders, EUA., Philadelphia.

Toma, B., B. Dufour, M. Sanaa, J.J. Benet, F. Moutou and P. Ellis, 1999. Applied Veterinary Epidemiology and the Control of Disease in Populations. AEEMA Edn., Maisons-Alfort, France.