Experiences in Mexico of the vaccination in bovines and goats, with *Brucella abortus* RB51 strain

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The control of brucellosis in Mexico since the 70's until 1998 was performed using cattle vaccination with S19 strain of *Brucella abortus* as a key tool. The use of strain RB51 was approved since 1998 and vaccination with strain S19 was gradually decreased until fully waived. The strain S19 was used again from 2005 and now both vaccines are applied equally to prevent brucellosis in Mexican cattle. The implementation of the RB51 at normal and reduced dose, was approved in 2006 for goats, an unusual event since it's not used anywhere in the world to prevent brucellosis in goats. Although at present the permission was withdrawn for use in goats. This paper describes and discusses the research done in Mexico and the world during the 1998-2010 periods, on cattle, and goat vaccination with strain RB51, to elucidate whether the use of this vaccine has been appropriate in Mexico.

Keywords Brucellosis, bovines, RB51, goats, Mexico

Experience of RB51 vaccination against goat brucellosis

In Mexico goats had an inventory in 2009 of 8 million heads. The annual production of 43, 129 tonnes of beef carcasses and 165, 699 liters of milk [1] Experiments were conducted in the murine model in order to know if the RB51 vaccine was able to protect against *B. melitensis.* It was found that BALB / c mice vaccinated with live RB51, , produced protection against challenge with virulent strains of *Brucella melitensis*. Experiments indicated that vaccinated mice were protected against *B. melitensis* 16M through cell-mediated and humoral immunity. These results encouraged the investigation of strain RB51 as an alternative vaccine against heterologous *Brucella* [2] It was found that that Rev 1 vaccine was superior to RB51 vaccine in mice against *B. melitensis* [3] The control program for brucellosis in Mexico, indicates the vaccination with Rev 1 strain of *B. melitensis*,. for goats, which induces a serological response very similar to natural infection so far. In Mexico the RB51 vaccine was tested in goats, expecting that by using live vaccines in the rough phase (R), which lack the surface antigens characteristic of *brucellae* in the smooth phase (S), did not induce cross-serological reaction with classical diagnostic tests that use S-phase antigens. Therefore it might be a good way to make a prophylactic program compatible with diagnostic tests [3,4] It is unknown if it is harmful for lactating goats or rams[5]

RB51 vaccine appears to have a low residual virulence in pregnant goats although due to the biases of these experiments, additional tests would be required. On the other hand, there is evidence that RB51 confers protection in goats against *B. melitensis* [6] Soberon (1997) reported that RB51 created a proper immune response against a challenge that infected 60% of the unvaccinated goats (RR values of this control versus the vaccine was 9.0 [P = 0.006]). However, it must be noted that the experimental protocol departs from others since animals had to be challenged twice before any became positive by standard serological tests This suggests that the first challenge was ineffective and it cannot be ruled out that this first challenge may have had an immunizing effect against the second challenge.[7]

Although there are experiments with contrary results, as Hernandez et al (2001) who compared RB51 with Rev 1. The number of animals in each group was different and very limited in some cases; contrary to Soberon (1987), Rev 1 but not RB51 conferred significant protection in this experiment (RRs of 8.0 [P = 0.05] and 2.4 [P = 11], respectively, for the comparisons with the non vaccinated controls[8].

Field research has been done in goats vaccinated with RB51 in Mexico, although many of the experiments performed have numerous methodological weaknesses and is commonly known that in order to recommend a vaccine controlled challenge experiments must be done first.[9-13]Mexico authorized the use of strain RB51 for goats, both the reduce and complete doses. However, the vaccine had a single presentation only varying the dose applied with 1 ml dose for the reduced and 2 ml for the full dose. Its use is no longer allowed.

It should be emphasized that RB51 is not used to protect against *B. melitensis* in goats or sheep anywhere else in the world.

In conclusion, the use of established and tested vaccines which have proven to be an effective tool for control and eradication for over 60 years as the Rev 1 which is used worldwide since the early 50 `s, with excellent results, should be preferred over those vaccines that have not been tested in well-controlled comparative experiments[14]

Experience of RB51 vaccination against bovine brucellosis

In Mexico cattle is a major activity with an inventory of 29 million heads, specialized in meat production in 1 million production units and 2.2 million cattle specialized in milk production at 789 thousand production units. The annual production of 1.5 million tonnes of beef carcasses and 10 billion liters of milk; occupying the 9th place worldwide in meat production and 15th in milk production. The production value was estimated from the carcass: 3538 million dollars and milk: 2, 846, millon dollars. With a value of live animal exports of 659 million dollars a year [1]
Brucellosis is a bacterial infectious disease that affects both humans and domestic animal species with a worldwide distribution and is considered as endemic in Mexico;and one of the main problems affecting livestock. This impact has not been possible to quantify because there are no reliable data on its prevalence in cattle. However, it is known to be widely distributed throughout the national territory, being the southeastern area the one with the highest incidence, followed by the center area of the country where the State of Mexico, Queretaro, Guerrero, Hidalgo and Michoacan states have a mean prevalence between 9 and 15%. Meanwhile northern Mexico has a lower incidence, most notably the state of Sonora for being declared free of brucellosis. This disease affects livestock farms resulting in decreased production, expressed in less kilos of meat for sale (17%), reduced number of replacement heifers, liters of milk produced are eroded by up to 30% and increased number of eliminated animals due to fertility problems, causing direct economic losses to producers [16].

The purpose of the different control programs are vaccination, prevention and eradication of brucellosis, particularly in developing countries. These programs are based on the socioeconomic conditions of each region in which the prevalence is usually high. Brucellosis must be controlled in cattle by application of live attenuated vaccines accompanied by animal health measures, because the vaccine itself is not a solution. Live vaccines stimulate a cell-type immune response which in turn is required to control intracellular living bacteria as *Brucella*. The ability to have memory cells after a first infection is the main step for a prolonged immunity. This is the basis of immunization [17-19].

The S19 vaccine has been produced and used in Mexico since 1951. The RB51 vaccine began to be used in the official campaign starting in 1997, leaving behind the use of S19 which even stopped being produced. Although it was returned to production from 2004, both vaccines are now being used interchangeably.
Strain RB51 is rough as it lacks the lipopolysaccharide O chain, this feature gives it an advantage because it does not induce the antibodies that are detected by official diagnostic tests, resulting in the differentiation of vaccinated from infected animals. Like the S19, in Mexico there is a calf dose and another dose for adult animals. The calf dose must contain $1 – 3 \times 10^{10}$ CFU, while the adult dose contains $1 – 3 \times 10^{9}$ CFU per dose and is applied to cows over 12 months of age. Another advantage of this vaccine is its low residual virulence and scientific studies show that abortions are not induced neither is excreted in milk. However, as mentioned later, re-vaccination can induce abortion and excretion though milk and vaginal discharge [20]

In recent years an inappropriate practice of vaccination against *B. abortus* has been performed in various states of Mexico (Aguascalientes, Queretaro, Hidalgo, Coahuila and Jalisco, among others). Immunizations are carried out because there is a large number of new cases in intensive farms. This type of farming is where vaccination against brucellosis is a common practice. Backyard farmers do not vaccinate repeatedly, instead they apply only one dose of vaccine during the control campaign.

Strains S19 and RB51 for cattle have proven their effectiveness if they are used with additional control measures as mentioned above. According to the characteristics of the herd one or the other (not both) must be chosen for brucellosis control, knowing the characteristics of each. There are places where heifers are immunized with S19 and subsequent vaccinated with RB51 or where heifers have been vaccinated and revaccinated with RB51 and subsequently immunized with S19.

Regarding the records related to the cattle vaccination in Mexico, there are two sources of information, first the number of vaccine doses marketed by the manufacturers and the other referring to the official reports of vaccination. Sales of 1'640, 000 doses of RB51 and 0 for S19, were reported in 2003. S19 was sold again from 2004 reporting 29,000 doses per 986,000 of RB51. In 2005 producers sold 686,000 doses of RB51 and 88,000 of S19. Meanwhile official records for 2004 show the application of slightly less than 1, 000,000 doses and during the first half of 2005 a figure close to 800, 000 doses. It is worth mentioning that a significant number of producers apply vaccines but do not notify the proper authorities, which is why the figures are somewhat uneven. Moreover, many animals were boosted and therefore the number of vaccines does not always correspond to the number of immunized animals [21].

2007 reported sales of 598, 000 and 105,000 doses of S19 and RB51; 959, 000 and 221, 000 doses were sold in Mexico of RB51 and S19 in 2008, and the year 2009, reported 864, 000 RB51 and 260,000 S19 doses sold [21] Vaccination of a large number of herds is not applied for whatever reason. For example, in the state of Aguascalientes in the period 1996-2004 the individual seroprevalence for brucellosis in vaccinated animals was 3.1%, which was

significantly higher than the individual prevalence among the non-vaccinated animals (1.9%). At first glance it may appear that the increased prevalence is associated with vaccination. However, we must consider that immunized herds are less than half in number than the unvaccinated, and that much larger herds, have a number of positive animals per herd of more than six times higher than the unvaccinated [22].

Additionally, it is convenient to consider the risk for most of the unvaccinated herds found in an infected area with which they exchange animals, goods and products. Therefore contagion should not be a casualty, but common and then restart an outbreak makes vaccination necessary again *post facto* and not as a precautionary measure. Other factors may also be involved, such as the failure to perform sampling and serological diagnosis, segregation, disposal and confinement of seropositive animals.

The reality is that there are animals over 12 months of age that have not been vaccinated as the Official veterinarians working for the Campaign do not regularly vaccinate calves, so that sometimes adult animals are immunized and those who have an abortion one or two months before delivery. Therefore it's not that vaccination does not work in some occasions, but that animals were already infected before for not being protected for a long time, especially when they confined with positive animals.

The Mexican Official Standard NOM-041-ZOO-1995 of the National Campaign Against Animal Brucellosis indicates that live vaccines should be used and that the vaccine is given in calf doses or if the animals have not been vaccinated when young, the reduced dose must be applied, but there is no indication that animals should be revaccinated [23]

An incorrect practice includes repeated vaccinations with RB51 vaccine, animals are revaccinated even up to 11 times at intervals of 3-6 months in some farms [24]. Applying so many boosters is not usual since regularly a cow in an intensive farm lasts an average of three deliveries, as elimination waste is premature because of the excessive stress (three daily milkings, lactotropine application, etc.) so that these animals have less than 3-4 boosters. In the states of Jalisco and Aguascalientes, some producers re-vaccinate with RB51 every 4-6 months as if it were a bacterin, not a live vaccine. In Queretaro, the Comarca Lagunera, Tizayuca, Guanajuato, etc., some farms advised by dairy companies, apply annual re-vaccination, even with calf-dose to animals older than 12 months with RB51, supposedly to have greater protection in adult cattle. As a result, it can affect fertility and cause bacterial shedding of the vaccinal strain in milk and vaginal discharge. Our research group has found that the vaccinal strain RB51 can be shed in milk and has been recovered from vaginal exudate from cows [25,26] and also DNA of this strain was detected in cows that aborted [19]. Although it has not yet been determined whether colonization and elimination by different routes are inherent in the vaccine strain or is due to misuse and repeated inoculation, which allows colonization and installation of the bacteria in tissues. Intracellular traffic was tested in order to determined if the RB51 strains isolated from milk had become virulent, finding that the vaccine strains were still avirulent and found in the lysosome within macrophages where they were destroyed [27]

The experiments conducted on pregnant cows showed that RB51 strain inoculated at a reduced dose has a very low residual virulence, despite being able to produce placentitis in animals inoculated intravenously with higher doses. It was found that RB51 caused one abortion out of 820 pregnant cows that had been vaccinated under field conditions with 1 billion CFU [28]. However, in Mexico cases of abortion in revaccinated cattle have occurred where there is evidence of RB51 presence [16]

Doing serological surveys in the affected populations facilitate taking decisions concerning which animals to cull, so it is important to consider that there must be access to a reliable laboratory to take a timely result and the implicit commitment acceptance of the result. Once results are obtained it is necessary to identify positive animals permanently to facilitate segregation or culling. In Tizayuca, Hidalgo which is a dairy area with endemic occurrence of brucellosis, producers have managed to reduce the incidence of brucellosis to less than 1% over eight years based on monthly serological surveys and removal of reactive animals at the end of their productive cycle, coupled with health management measures [29,30]

The efficacy of RB51 vaccine was evaluated in a dual-purpose herd under tropical conditions with a positive rate of 5% with isolates of *B. abortus* from milk samples. The vaccinated and boostered groups were integrated by 88 females each. No reactive animals were removed or segregated from the population. Three new cases of infection occurred during the 18 months of monitoring in the unvaccinated group so that the rate of positive reactors to serological tests increased to 12.5%. In the vaccinated group this rate was maintained at 0% due to 100% protective efficacy provided that the strain RB51 to the total vaccinated population [31]

It is known that the immune response against microorganisms of the genus *Brucella* is Th1 cell type, which is stimulated only with live vaccines. However, there are also scientific evidence that the immune response is down regulated when antigen stimulation is constant (as in chronic diseases such as tuberculosis) and can result in a shift towards a Th2 humoral response [32] which is not effective for protection or elimination of brucellosis. A leading risk factor for the presentation of the disease is replacement animals, so they should be monitored before, during and after admission to exploitation. These animals must be negative to serological tests and also come from herds free of brucellosis. When replacements are generated inside the production unit, it should be taken into account the phenomenon of "immunological silence" which is the incubation period of the disease, as 20% of the daughters of seropositive cows remain seronegative from birth until about to give birth. Inadequate vaccination, especially the

repeated booster every six months or every year in cows with a reduced dose and normal dose of RB51 lately (used for heifers) or S19 is a common practice in Mexico; although it has no scientific basis and to which can be attributed the shedding of *Brucella* in vaginal exudate and milk from apparently healthy cows that aborted, and to encourage the presence of cows anergic and immunotolerant to brucella. Our research group has found disturbing facts that indicate improper use of vaccines in cows vaccinated with RB51. Results include detection of cows with anergy, isolation of RB51 from milk and vaginal exudate, demonstration of boosters as the cause of abortions and other alarming evidence that is isolation of field and smooth phenotype *Brucella abortus* in yearling cows that calved normally and were seronegative for brucellosis.

RB51 apparently does not interfere with serological rivanol and card tests, as no antibody response occurs against the O chain, so any animal that presented a positive reaction to these tests should be regarded as infected. However, 17% animals previously vaccinated and revaccinated with RB51, were positive to the card test, but negative to rivanol for several months [18] Diagnosis is uncertain during the time after immunization, because the control program in Mexico provides that animals found as positive to the card test after vaccination or revaccination with RB51 must be regarded as infected. To explain this post-vaccination response it is believed that the primary stimulation is activated by RB51 vaccination, and then because of the extensive spread of *Brucella* in endemic areas, cows may develop a secondary response, so occasionally animals that have antibodies and are positive for official testing are found because they are in contact with the field pathogen. Nonetheless, the immune system can control the infection since they are negative to IDR and bacterial isolation tests, which indicate infection. Therefore confirmatory tests are indispensable to differentiate vaccinated from infected animals, such as the IDR and competitive ELISA.

Since S19 and RB51 vaccines are alive and virulent for humans, it poses potentially infectious risk for people. Additionally, the fact that the rough strain RB51 vaccine is resistant to rifampicin must be considered. In case of accidental infection with the vaccine strain, it could be happen that the diagnostic tests available for diagnosis in humans are negative (recall that the rough LPS does not induce the formation of the same antibodies that are formed with smooth strains), in addition that rifampicin is a drug of choice for the treatment of human brucellosis, to which strain RB51 is resistant.

The use of live attenuated S19 and RB51 in cattle has been very useful, but vaccination is only a tool that alone does not allow control of brucellosis. Health management measures and timely diagnosis are needed to control the disease. This was demonstrated by an experiment conducted in Tizayuca Hidalgo, which undertook a prospective longitudinal study in two dairy cattle herds, one with low prevalence including 538 cows and one with high prevalence in 612 cows. Animals were vaccinated with *Brucella abortus* strain RB51 on two occasions with a 90-day-interval at doses of 1×10^9 cfu / ml. Samples were taken from all animals every 30 days for a period of seven years. Blood samples were obtained from the ventral coccygeal vein. The card test was performed for the identification animals seropositive to *B. abortus* and then the rivanol test was performed to confirm this result. Sera were considered rivanol positive when they presented complete agglutination reaction in any of the dilutions, 1:25-1:400. Prevalence and apparent incidence of brucellosis were assessed during the study, according to the presence of antibodies against *B. abortus*. Seroprevalence of brucellosis in the two farms in Tizayuca at baseline were 15.3% and 8.2% respectively. In samples from days 90, 120 and 150 incidence was 34%, 19% and 21% respectively, reaching 52% at day 270. During the first 360 days of study seropositive cows were not removed from the farm, but were located in a specific pen. From days 360 to 960 positive animals started being culled, depending on the serum antibody titer in rivanol. Animals with titers of 1:100, 1:200 and 1:400 were eliminated gradually; however, cows with titers between 1:25 and 1:50 were left in the special yard and were culled only if titers increased the next sampling. This management yielded incidence lower than 2% during this period. Later, elimination of seropositive animals was stopped during days 990 and 1830 so the monthly incidence of 2% was not obtained again, reaching 9.9% of seroprevalence. Positive cows were culled again for what incidence was less than 2% during the period 1860 to 2580 days. [29, 30]

Another study implemented a management program for bovine brucellosis in infected herds in five dairy farms from Juventino Rosas, Guanajuato. During the 12-month study there was an average of 400 Holstein cows at each sampling, herd 1 with 25 cows, herd 2 with 37, herd 3 with 85, heard 4 with 81 and herd 5 with 172 cows. Animals were vaccinated subcutaneously at the beginning of the study with RB51 (N = 200) or S19 (n = 200) at a reduced dose for both strains. Once immunized, all animals were sampled every 30 days for a period of 360 days (12 months). The card test was performed to identify animals seropositive to *B. abortus* and then the Rivanol and radial immunodiffusion tests were done to confirm this result. Initial and final prevalence and apparent incidence of brucellosis were assessed during the study, according to the presence of antibodies against *B. abortus.* Initial brucellosis seroprevalence of herds 1, 2, 3, 4 and 5 were 16%, 30.3%, 21.1%, 18.3% and 13.9% respectively, having an initial mean seroprevalence of 19.9%. At the end of the study the final seroprevalence was determined for each farm with the following results: herd 1 – 8.3%, herd 2 – 24.3%, herd 3 – 16.3%, herd 4 – 13.3% and herd 5 – 12.9%, with a final seroprevalence of 15%. However, incidence during the 12-month study behaved as follows, for herd 1, 2, 3, 4 and 5 it was 0%, 16.2%, 5.3%, 1.3% and 4.5% respectively, of which these new cases corresponded to 59% cows vaccinated with RB51 and 41% of animals immunized with S19. [33]

In Mexico, the Brucellosis control results remain limited, mainly due to inadequate vaccination, poor immunization coverage and the lack of public health actions, such as early diagnosis, segregation especially at birth and after this and culling of positive animals.

References

[1] Servicio de información y estadística agroalimentaria y pesquera (SIAP) 2010. SAGARPA, México. Available at: http://www.siap.gob.mx/index.php?option=com_content&view=article&id=21&Itemid=330. Accessed June 7, 2010

[2] Jiménez de Bagüés MP, Elzer PH, Jones SM, Blasco JM, Enright FM, Schurig GG, Winter AJ.Vaccination with *Brucella abortus* rough mutant RB51 protects BALB/c mice against virulent strains of *Brucella abortus, Brucella melitensis*, and *Brucella ovis*. *Infect Immun*. 1994; 62:4990-4996.

[3] Hamdy MER, El-Gibaly M, Montasser M. Comparison between immune responses and resistance induced in BALB/c mice vaccinated with RB51 and Rev. 1 vaccines and challenged with *Brucella melitensis* bv. 3. *Vet Microbiol* 2002; 88: 85-94

[4] Díaz R, Jones LM, Leong D, Wilson JB. Surface antigens of smooth *Brucellae*. *J Bacteriol*; 1968; 96: 893-901;

[5] Blasco MJM. Vacunas clásicas y de nueva generación frente a la brucelosis en pequeños rumiantes. IN: IV Foro Nacional de Brucelosis. November 26 and 27, 2007. Facultad de Medicina Veterinaria y Zootecnia, Cuidad Universitaria; D.F pp 118-155

[6] Schurig G. Rough Brucella vaccines: *Brucella abortus* RB51 and analogue vaccines for *B. melitensis*. WHO Meeting on development of new/improved brucellosis vaccines. Geneva. Switzerland. 1997.

[7] Soberón MA. Protección conferida por la vacuna RB51 de *Brucella abortus* en cabras expuestas a la infección experimental por *Brucella melitensis*. Master of Science thesis, Universidad Nacional Autónoma de México 1997.

[8] Hernández L, Ochoa D, Díaz E, Córdoba L, López M, Ontiveros C. Conferred protection for RB51 vaccine in goats, 54th Annual Brucellosis Research Conference. Nov 10–11. St. Louis, 2001.

[9] Villa R, Díaz AE, Alicia Soberón M A, Laura Hernández AL, Suárez GF. Presencia de aborto y mortinatos en cabras inmunizadas contra brucelosis con las vacunas RB51, *rfb*K *y* Rev 1. *Téc Pecu. Méx*. 2008; 46: 249-258

[10] Soberon-Mobarak A, Diaz-Aparicio E, Torres-Armenta J, Adams LG, Suarez-Güemes F. Absence of shedding of two *B. abortus* strains in goats after vaccination with live vaccines *Vaccine.* 2000; 18: 3018-3020.

[11] Martínez HDI, Abeledo GMA, Moreno, M. M.; Romero BDE, Rivera REL, Zilli, D. E.; Rodríguez CMA, Peniche CAJE, Acosta MEA, Luna MJE. Evaluación de la vacuna rev-1 de *Brucella melitensis* en rebaños caprinos de Tenextepec, Municipio de Perote, *Ver. Rev. Salud Anim*. 2000; 23: 91–96.

[12] Martínez HDI, Abeledo GMA, Rodríguez CMA, Jácome RJ A, Rivera REL, Vallecillo MAJ, Romero BRM, Romero BDE, Acosta MEA, Luna MJE. (): Prevalencia de Brucelosis caprina y su relación con la humana en Tenextepec, Municipio de Perote, Veracruz, México. *Rev. Salud Anim*. 2001[a]; 23: 160–164.

[13] Martínez HDI, Abeledo GMA, Rodríguez CMA, Bautista BR, Rivera REL, Vallecillo MAJ, Alpírez MM, Acosta MEA, Luna MJE. Aislamiento de *Brucella melitensis* biovar 1 en leche de cabras de la comunidad de Tenextepec, Municipio de Perote, Veracruz. *Rev. Salud Anim*. 2001b; 23: 123–127.

[14] Grillo MJ, Monreal D, González D, Marín C, López-Goñi I, Mainar-Jaime RC, Moreno E, Blasco JM. Rough vaccines in animal brucellosis:Structural and genetic basis and present status. *Vet. Res*. 2004; 35: 1–38

[15] Luna, J.M., T.C. Mejía.. Brucellosis in Mexico: current status and trends *Vet Microbiol* 2002; 90:1-4 :19-30.

[16] Milian SF, Garcia CL, Anaya AE. Distribución de enfermedades de importancia económica de los animales domésticos en México 1990-2004. *Programa Nacional de Epidemiología*. INIFAP 2004.:26-34

[17] Blasco, J. M. Existing and future vaccines against brucellosis in small ruminants. *Small Ruminant Research* 2006: 1-2: 33-37.

[18] Cassataro, J., C. A. Velikovsky, et al. Improved immunogenicity of a vaccination regimen combining a DNA vaccine encoding *Brucella melitensis* outer membrane protein 31 (Omp31) and recombinant Omp31 boosting. *Clin. Vaccine Immunol.* 2007; 14.7: 869-874.

[19] Olsen, S. C., Stoffregen, W. S. Essential role of vaccines in brucellosis control and eradication programs for livestock. *Expert.Rev.vaccines*. 2005; 6: 915- 928

[20] Baldwin, C. L. and R. Goenka Host immune responses to the intracellular bacteria *Brucella:* does the bacteria instruct the host to facilitate chronic infection?. *Crit. Rev. Immunol*. 2006; 26. 5: 407-442.

[21] Betancourt MX. Situación actual y perspectivas de la Campaña contra Brucelosis en México. Taller sobre el diagnóstico de las principales enfermedades en caprinos"FMVZ-UNAM May 27, 2010

[22] Barquero-Calvo, E., E. Chaves-Olarte, et al. Brucella abortus uses a stealthy strategy to avoid activation of the innate immune system during the onset of infection. *PLoS ONE*. 2007; 2. 7: e631.

[23] Norma Oficial Mexicana NOM-053-ZOO-1995. Requisitos mínimos para las vacunas, antígenos y reactivos empleados en la prevención y control de la brucelosis en los animales. Available at: http://www.sagarpa.gob.mx/Normas-oficiales. Accessed June 7, 2010

[24] Bustamante, S.J., F. Salazar, H., E. Díaz, A., C. Manzano, C., *et al.* Estudio bacteriológico y serológico de brucelosis en vacas revacunadas con dosis reducida de cepa S19 de *Brucella abortus*.*Tec.Pecu.Mex*.2000; 38. 1: 35-42

[25] Leal-Hernandez, M., E. Diaz-Aparicio, et al.. 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* 2005; 28; 1: 63-70.

[26] Fuentes, D.M.D., I. Vitela, B. Arellano-Reynoso, R. Hernández,, F. Morales, C Cruz-Vázquez, F., Suárez-Güemes, E. Díaz-Aparicio.. Presence of *Brucella abortus* vaccinal strain RB51 in vaginal exudates of aborted cows. *Res. J. Dairy Sciences;* 2007; 1, 1: 13-27.

[27] Arellano-Reynoso, B., E. Diaz-Aparicio, M. Leal-Hernandez, L. Hernandez, and J. P. Gorvel, 2004. Intracellular trafficking study of a RB51 *B. abortus* vaccinal strain isolated from cow milk. *Vet. Microbiol;* 98. 3-4:307-312.

[28] Palmer, MV, N.F. Cheville, A.L.E. Jensen. 1996. Experimental infection of pregnant cattle with the vaccine candidate *Brucella abortus* strain RB51: Pathologic, bacteriologic and serologic findings. *Vet. Path*. 33: 682-691
[29] Herrera López Enrique. Programas de control en Hatos bovinos infectados con diferentes prevalencias de Brucelosis. *Memorias del IV Foro Nacional de Brucelosis*, FMVZ, UNAM, 26-27 de noviembre de 2007, D.F, México.
[30] Herrera E, Palomares G, Diaz E. Milk Production increase in a Dairy Farm, under a six-year Brucellosis Control Program. Animal Biodiversity and emerging diseases: *Ann.N.Y.Academ.Sci*. 2008; 1149:296-299
[31] Flores, C.R. Vacunas contra brucelosis y la vacunación en México. *IV Foro nacional de brucelosis* November 26 and 27, 2007 Facultad de Medicina Veterinaria y Zootecnia, Ciudad Universitaria; D.F
[32] Kidd, P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern Med Rev* 2003,8, 223-246.
[33] Herrera LE; Luna AM; Banda RV; Socci EG; Guzmán RC; Hernández AL; Córdova LD Manejo de hato infectado por brucelosis bovina en el estado de Guanajuato, Mexico *XXXI Congreso Nacional de Buiatria*. August 9 – 11, 2007 Centro de convenciones Acapulco, Guerrrero