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Study of Brucellosis Incidence in a Bovine Dairy Farm Infected with *Brucella abortus*, Where Cattle Was Revaccinated with RB51

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Abstract: A prospective longitudinal study was performed of brucellosis' monthly incidence during a period of six years, in a bovine dairy farm revaccinated with RB51 and endemic presentation of brucellosis, monthly serologies were done to the total amount of cows (average of 340). Prevalence at the beginning of the study was 15.3%. All negative animals were vaccinated with RB51 reduced doses and revaccinated. Card Test (CT) was executed and the positive sera were tested by rivanol. Sampling on day 150 showed incidence of 21%. During the first 360 days of the study, the cows that were positive to serology were not eliminated. During the period between 390 to 960 days, positive animals were eliminated, obtaining with this type of handling, incidences below 2%. Between days 990 to 1830 we returned to the criteria of not eliminating positives and therefore monthly incidences were 2%. From day 1830 on, all seropositive cows were eliminated with incidences during day 2160 and 2190, last day of our study, of 0.5 and 0.25%, respectively. The conclusion that we arrived at is that to have an efficient control of brucellosis, a vaccination program appropriate for epidemiological conditions of brucellosis in the region is required.

Key words: *Brucella abortus*, brucellosis, incidence, RB51, cows

Introduction

Brucellosis is an infectious and contagious disease that affects humans as well as domestic animal species. It causes economic loss due to abortions, lengthening of the period between calvings, loss of genetic lines, infertility and sterility, in bovines, milk production is reduced by up to 30% (Acha and Zyfres, 1986).

Brucella abortus is an intracellular pathogen that survives inside macrophages in order to avoid the hostile environment. During the course of infection the bacteria remains within the cells and therefore these are the ones responsible for the destruction of the pathogen or be themselves destroyed so that other mechanisms can gain access to the *Brucella* and eliminate it, for example antibody mediated means (Golding *et al*., 2001). In Mexico, beginning in 1998, RB51 vaccine was used in control programs within the official campaign (Castell, 1998).

B. abortus RB51 is an attenuated rough strain stable vaccine, derived from the virulent strain 2308. This vaccine strain is incapable of producing the polysaccharide O chain (Cheville, 2000; Vemulapalli *et al*., 2000). The lack of this chain allows us to use this vaccine without the production of antibodies that may interfere with the serological diagnosis of this disease. Due to this fact, any positive reaction to official tests of the animals is diagnosed as infected (Uzal *et al*., 2000).

Nevertheless, this statement is valid under the circumstances found in countries with low prevalence of the disease; such is the case of the United States, where brucellosis is practically

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eradicated and incidence is very low. In contrast, in Mexico, the disease is endemic and the circumstances are very different. It has been observed that bovines without infection, either vaccinated or revaccinated with strain RB51, come out positive to serological testing several months post-immunization, which causes diagnosis confusion (Díaz-Aparicio *et al*., 2004; Leal-Hernández *et al*., 2005).

Serological samplings in affected populations facilitate taking decisions for the culling of animals and therefore a reliable laboratory should be accessible, in order to have opportune results with the implicit acceptance of the results. Once the results are available, it is necessary to identify positive animals in a permanent manner in order to facilitate segregation or elimination of the animals from the farm (Luna and Mejía, 1998). The objective of this study was the determination of monthly incidences during a six-year period in a bovine dairy herd infected with brucellosis and revaccinated with RB51.

Materials and Methods

Animals

Serological follow-up was performed of a bovine dairy herd with cows of Holstein breed, located in the Industrial Agricultural and Livestock Complex of Tizayuca, Hidalgo, Mexico. In this dairy basin, brucellosis has an endemic status and this dairy had *B. abortus* biotype 1 infection demonstrated by isolation.

Tizayuca, Hidalgo, Mexico, dairy basin has around 25,000 Holstein breed cows, specialized in milk production and distributed in 126 dairies; throughout the basin there is 20% brucellosis prevalence.

The dairy herd that was studied is within the dairy basin, its handling system is intensive, the entrance to the dairy has sanitary trenches for vehicles as well as persons, four pens with individual stalls, feed bunks and drinkers, feed is integrated by a mixer car and the animals are milked three times per day. Seropositive animals to brucellosis are identified and grouped in a specified pen where they are no longer inseminated and when their milk production is reduced or they have any health problem they are gradually eliminated from the dairy.

Vaccination

At the beginning of the study (day zero) the total amount of negative animals were vaccinated with RB51 at a 2 mL reduced dose at a concentration of 3×10^9 cfu mL^{-1}, application was subcutaneous in the left axillary zone.

Revaccination took place at day 90 and every 360 days during the six years that the study lasted, with the same reduced doses of RB51 and the same route detailed previously.

Samplings

Serological samplings were performed for all the animals every 30 days during a 2190 days period (72 months), the monthly average of worked cows was 340. Blood samples were taken from the ventral coccygeal vein.

Milk and vaginal samples were taken, from the 37 seropositive cows that were detected at experiment day 0.

Serology Studies

To identify animals that are seropositive to *Brucella*, all animals were tested by Card Test (CT) and positive results were confirmed by rivanol (Alton *et al*., 1988). By rivanol test, sera were considered positive when they presented an agglutination reaction to any of the dilutions from 1:25 to 1:400.

Bacteriology Studies

Milk and vaginal exudate samples were inoculated in duplicate in TSA and Farrell (1974), incubated during 10 days with 10% CO_2 at 37°C. Conventional methodology was used to identify isolated strains (Alton *et al*., 1988).

Epidemiological Studies

A prospective longitudinal study was performed estimating monthly brucellosis incidence and prevalence during a period of six years, according to the presence of *B. abortus* antibodies detected by CT serological and rivanol tests (Anonymous, 1978).

Results

Brucellosis seroprevalence in the dairy when the study began was 15.3% (37/241). Incidence by samplings on days 90, 120 and 150 was: 34.5% (56/162), 19.3% (30/155) and 20.9% (30/143), respectively (Table 1).

Bacteriology studies performed on vaginal exudates and milk samples gave as a result isolation and identification of four strains of *B. abortus*, biotype 1 with smooth phenotype.

During the first 360 days seropositive cows of the study were not eliminated from the dairy, nevertheless they were placed in a pen specific for them.

Table 1a: Monthly prevalence and incidence in bovine herd infected for brucellosis, where cattle was revaccinated with RB51

Day	Animals No.	Negatives No.	Positives No.	New positives No.	Prevalence (%)	Incidence (%)
0	241	204	37	0	15.35	0.00
30	253	225	28	1	11.07	0.44
60	254	216	38	16	14.96	7.41
90	255	162	93	56	36.47	34.57
120	255	155	100	30	39.22	19.35
150	248	143	105	30	42.34	20.98
180	253	141	112	12	44.27	8.51
210	266	141	125	15	46.99	10.64
240	270	139	131	8	48.52	5.76
270	254	122	132	9	51.97	7.38
300	267	135	132	10	49.44	7.41
330	250	123	127	2	50.80	1.63
360	254	134	120	2	47.24	1.49
390	250	159	91	3	36.40	1.89
420	243	156	87	3	35.80	1.92
450	245	175	70	0	28.57	0.00
480	240	199	41	5	17.08	2.51
540	284	249	35	1	12.32	0.40
570	380	350	30	0	7.89	0.00
600	372	348	24	0	6.45	0.00
630	387	371	16	0	4.13	0.00
660	383	367	16	1	4.18	0.27
720	395	378	17	2	4.30	0.53
750	375	356	19	1	5.07	0.28
810	380	361	19	0	5.00	0.00
870	364	350	14	4	3.85	1.14
900	384	376	8	5	2.08	1.33
930	387	382	5	4	1.29	1.05
960	390	380	10	8	2.56	2.11
990	389	378	11	2	2.83	0.53
1050	402	394	8	2	1.99	0.51
1080	400	390	10	0	2.50	0.00
1140	398	392	6	1	1.51	0.26
1170	400	394	6	1	1.50	0.25

Table 1b: Continued

Day	Animals No.	Negatives No.	Positives No.	New positives No.	Prevalence (%)	Incidence (%)
1290	395	391	4	0	1.01	0.00
1380	397	396	1	5	0.25	1.26
1440	380	374	6	0	1.58	0.00
1470	362	356	6	3	1.66	0.84
1530	360	351	9	9	2.50	2.56
1560	363	345	18	1	4.96	0.29
1590	357	338	19	1	5.32	0.30
1650	360	346	14	5	3.89	1.45
1680	335	316	19	1	5.67	0.32
1710	330	314	16	8	4.85	2.55
1740	326	305	21	2	6.44	0.66
1770	320	297	23	3	7.19	1.01
1800	342	314	28	4	8.19	1.27
1830	324	292	32	4	9.88	1.37
1860	375	375	0	6	0.00	1.60
1890	390	390	0	3	0.00	0.77
1920	405	402	3	2	0.74	0.50
1950	395	392	3	2	0.76	0.51
1980	381	380	1	0	0.26	0.00
2010	383	379	4	3	1.04	0.79
2040	389	384	5	2	1.29	0.52
2070	360	357	3	2	0.83	0.56
2100	360	356	4	2	1.11	0.56
2130	403	398	5	3	1.24	0.75
2160	400	397	3	2	0.75	0.50
2190	404	401	3	1	0.74	0.25

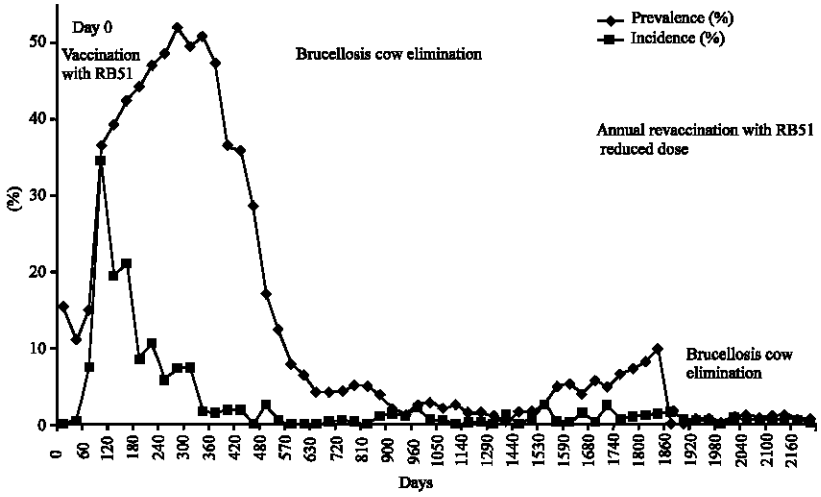

Fig. 1: Monthly prevalence and incidence in dairy cow herd infected with brucellosis, where cattle was revaccinated with RB51

During the interval between 360 and 960 days animals began slowly to be eliminated, taking into consideration the rivanol antibody serology titers, the animals with 1:100, 1:200 and 1:400 titers were slowly eliminated; nevertheless, cows with 1:25 and 1:50 were left in the designated pen and only if during the next sampling titers went up they were eliminated. This handling resulted in incidences below 2% during this period.

But, from day 990 to 1830, the non-elimination of positive cows was implemented again and monthly incidences were around 2%.

Beginning day 1830, all seropositive cows were eliminated from the dairy and incidences that were observed were 0.5% (2 of 401) on day 2160 and 0.25% (1 of 401) at day 2190. The study was ended on this day (Fig. 1).

Discussion

Vaccination strain RB51, has been used in Mexico, since 1998, very successfully and it slowly has been replacing strain S19. Discussions began since 2005, on how well strain RB51 worked, so strain S19 started gaining ground again. At this time both vaccines are being used in bovines indistinctly.

These discussions on RB51 vaccines occur mainly in endemic zones of our country, where after vaccination, cows are found positive to card test and rivanol tests with low titers. The reaction is transitory since it becomes negative after two or three months and this is attributed to the immune response facing exposure to a field strain of *Brucella* that is favorably resolved by the immune system. This should speak highly of RB51 vaccination, but it is not so because the vaccines are sold under the indication that no post-vaccination reactions occur and if there are positive animals to card tests, these undoubtedly are infected animals. But this claim happens only in zones with null or low prevalence and it is not the case in endemic areas, where animals are constantly challenged.

This response has been experimentally documented by Stevens and Olsen (1996) who demonstrated that RB51 immunized cows; later infected with *B. abortus* strain 2308, produce small amounts of IgG but not IgM. Also Cloeckaert *et al*. (2002) observed that strain RB51 produces low levels of an antigen that is similar to M; nevertheless, subsequent exposures to *Brucella* has some animals responding to chain O and are detected by conventional tests.

In controlled experiments, animals vaccinated with RB51 strain developed titers against *Brucella* after challenge with virulent 2308 strain and they have constant or decreasing titers until calving (Cheville *et al*., 1993; Olsen, 2000; Bagnat and Maneti, 2002).

Under field conditions, with endemic presence of brucellosis, our research group has found that when animals are naturally challenged, there is a recurrent serological response that is transitory (Leal-Hernandez *et al*., 2005; Herrera, 2002; Cantú *et al*., 2005). This response is derived from the primary immune response against *B. abortus* due to vaccination with RB51 and later challenge due to the extensive dissemination of field strains in endemic zones. Cows then develop a secondary response when they come into contact with these field strains. Antibody presence demonstrates that the animals have had contact with field strains, but their immune response was capable of controlling the infection (Díaz-Aparicio *et al*., 2004).

These data contrast with what Lord *et al*. (1998) found, since under these conditions of high prevalence (39%), bovines were vaccinated and revaccinated with RB51, at 5×10^9 cfu doses and later revaccinated with S19, finding that all vaccinated animals with RB51 resulted negative to conventional serological tests notwithstanding the natural challenge by field strains. These field results obtained with RB51 vaccination (Lord *et al*., 1998), are difficult to analyze and compare with those made under controlled conditions (Cheville *et al*., 1993; Olsen, 2000; Bagnat and Maneti, 2002). Under a more controlled scheme, Moriyón *et al*. (2004) observed that with this type of experiments, protection conferred by RB51 is useful under a moderate challenge, but when challenge is increased, RB51 is insufficient to prevent infection.

Moriyón *et al*. (2004) mentioned that the experiment by Lord *et al*. (1998) has certain defects in the selection of animals free from *Brucella*. Those animals belonged to infected herds and not free from brucellosis herds and were only selected based on negative serology. This method doesn't take into consideration young animals that acquire latent infections that are not manifested in serology tests and this introduces a bias in the selection of vaccinated groups. Also it is important that we highlight that

none of the animals vaccinated with RB51 became positive to the serology tests even though they were in contact with a field strain. Even though the field studies are less reliable, the results should be taken with caution when they openly contradict the results of controlled studies (Moriyón *et al*., 2004).

In the dairy basin where this study took place, brucellosis has an endemic status and high prevalence. Bovines that enter the basin are vaccinated and revaccinated with RB51 and besides each year they are revaccinated with a reduced dose of RB51. This procedure exists since the nineties but the vaccines that were used were S19 (Aparicio *et al*., 2003; Bustamante *et al*., 2000, González *et al*., 2006). Transitory post-vaccination responses are common in this dairy basin, therefore the non-official diagnosis criteria is that a cow is considered as having brucellosis only when it comes out positive to card tests and rivanol at titers 1:100 or higher, if they have lower titers to the rivanol test, it is repeated every month until the titers disappear or increase.

Revaccination is recommended by the Mexican Official Standard (1996) but on condition it is done only in endemic zones that have high prevalence using reduced doses and what is most important is that revaccination is done only one time. Repeated revaccination is not recommended since brucellosis vaccines are live and only one dose is capable of eliciting a solid response that is sufficient to protect the animal during all of its productive life (Diaz-Aparicio *et al*., 2004; Elzer *et al*., 1999; Nicoletti, 1990), that in Mexico is around 3.16 lactations (Valdespino, 1999; Vitela *et al*., 2004).

Revaccination is not necessary since RB51 vaccine induces a good cell type immune response, where Th1 and early gamma interferon production have a prominent role in protection (Elzer *et al*., 1998). Also during its intracellular transit RB51 is destroyed in the phagolysome and the resulting peptides are presented to the T cells through the main histocompatibility complex (Arellano *et al*., 2004). What has happened is that repeated RB51 revaccination has promoted the presentation of anergic animals (Leal, 2003).

Undoubtedly vaccination is an important tool to avoid the transmission of brucellosis amongst animals. Nevertheless, a control program in dairies with high prevalence should be integral to avoid the dissemination of the disease. What is ideal is that before beginning vaccination the infected animals be known by periodic serology controls, also, an epidemiological surveillance plan should be instrumented as well as a sanitary handling program.

When comparing incidences, between periods, when reactors were eliminated and when they were not, we can observe that there is a marked difference between both incidences and thus we conclude that this type of handling is essential to control brucellosis in an infected herd.

The increase of new cases during the first year of study (at 360 days) is attributed to seropositive animals that were not eliminated from the dairy. The number of positive animals that were found in the herd was considerable, since prevalence reached up to 51.9% (132/254) at day 270. Elimination of animals seropositive to brucellosis was performed according to their production and reproduction stage. We must underline that in Mexico the main causes for culling are urogenital problems, especially low production and infertility (Vitela *et al*., 2004).

Presence of seropositive animals represents an important risk factor and therefore control programs should be implemented that take into consideration the use of quarantines and continuous serological monitoring, in order to identify opportunely cows that are beginning with the disease, thus avoiding that these animals disseminate the disease to others at the time of calving or abortion, besides the continuous gradual dissemination.

During the incubation period of the disease it is very difficult to perform serological diagnosis. In fact, it is known that 20% of daughters of reactor dams may remain seronegative since birth until they are about to calve, after that, they become seropositive, notwithstanding they may have been vaccinated as calves, thus making it necessary to follow-up daughters of reactor dams during 24 months. It has also been recommended that all those cows that have aborted or have reproductive problems have serology tests in order to obtain a negative result before insemination, otherwise they should not be includes in the process (Luna and Mejía, 1998).

The most appropriate parameter to determine the degree and progress of infection in a herd is incidence, in this study we performed a monthly determination during six years finding that vaccination notably reduced brucellosis incidence and during those times when seropositive animals were eliminated, incidence reduction was reinforced, especially during the first three years.

The conclusion is that in order to have an efficient brucellosis control, besides a vaccination program that is appropriate to the epidemiological conditions of brucellosis in the region, continuous serological monitoring should be implemented to opportunely identify cows that are beginning with the disease in order to eliminate contagion sources, taking also into consideration the gradual elimination of positive animals.

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