SEROLOGICAL RESPONSE TO AN INDIRECT AND A COMPETITIVE ELISA IN HEIFERS VACCINATED WITH *BRUCELLA ABORTUS* STRAIN 19

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Abstract

SEROLOGICAL RESPONSE TO AN INDIRECT AND A COMPETITIVE ELISA IN HEIFERS VACCINATED WITH *BRUCELLA ABORTUS* STRAIN 19.

The different serologic techniques for bovine brucellosis diagnosis have different abilities to detect antibodies after vaccination with *Brucella abortus* strain 19. The humoral response in heifers vaccinated with *Brucella abortus* strain 19 was evaluated by using several serologic techniques. In the experimental field of INTA, Pilcaniyeu, Rio Negro province, sixteen 5 months old heifers were vaccinated subcutaneously with a standard dose (2ml, containing $20x10^9$ to $10x10^9$ living organisms) of *Brucella abortus* strain 19. Sera from all the heifers were obtained on 18 occasions (one 87 days before vaccination, one immediately before vaccination and on 16 occasions after vaccination, during 488 days) and analyzed by buffered plate antigen test, rose bengal test, standard tube agglutination test, 2-mercaptoetanol test, complement fixation test, indirect ELISA, and competitive ELISA. Prior vaccination, 100% of the heifers gave negative results in all the techniques used, while 100% of them gave positive reaction in the first sampling after vaccination to all the techniques, with the exception of standard tube agglutination test that showed agglutinating titters of 1/100 or higher (positive threshold) in only 71.4% of the heifers. The indirect ELISA technique showed a reducing percentage of positive animals up until 316 days after vaccination, after which positive results were obtained.

The competitive ELISA gave positive results in a variable number of heifers up to 253 days after vaccination when 100% of the sera were negative to this technique. Buffered plate antigen test was the technique that gave positive results for a longest period, being 100% of the animals negative to this technique at 450 days after vaccination. The other serological techniques assayed gave positive results during variable periods of time, intermediate between standard tube agglutination test and buffered plate antigen test. Although the present results were obtained from a limited number of animals, they clearly show that the diagnosis of bovine brucellosis in animals older than 18 months, with the techniques used here is not interfered by the residual antibodies after vaccination with strain 19 at 5 months of age.

1. INTRODUCTION

Bovine brucellosis is a zoonotic infectious disease produced by *Brucella abortus* [1]. Although the resistance to bovine brucellosis is essentially cellular (Sutherland, 1980), the disease generates the production of antibodies that albeit do not protect against the infection, are very useful for the diagnosis of the disease. These antibodies are of the type IgG_1, IgG_2 and IgM and are detectable by different serological techniques [2].

In Argentina, the serologic techniques more frequently used for diagnosis of bovine brucellosis are rose bengal test (RB), buffered plate antigen test (BPA), tube agglutination test (SAT), 2- mercapto ethanol test (2ME) and, less frequently, complement fixation test (CF). Calfhood vaccination with strain 19 (S-19) is compulsory between the age of 3 and 10 months of age (Resolución 1269/93 del Sercivio Nacional de Sanidad Animal, 16-11-93).

The S-19 is a live vaccine which may produce persistent antibodies that make difficult the interpretation of diagnostic serological tests [3]. The different serologic techniques for diagnosis of bovine brucellosis have different abilities to detect antibodies after vaccination with *B. abortus* S-19 [3, 4].

The information available about the persistence of antibodies after vaccination with S-19 in heifers, measured with different serologic techniques (particularly with ELISA) is scanty and frequently

contradictory. The objective of this study, therefore, was to evaluate the serological response in heifers with different serological techniques after S-19 vaccination.

2. MATERIAL AND METHODS

2.1. Animals

Sixteen Hereford heifers born and reared under field conditions on a farm without clinical and serological evidence of bovine brucellosis during the last 10 years in the Pilcaniyeu Department, Rio Negro Province, Patagonia, Argentina, were used.

2.2. Vaccination

All the heifers were subcutaneously vaccinated at 5 months of age with standard dose (10×10^9 to 20×10^9 living organisms per dose of 2 ml) of a commercial S-19 vaccine (Laboratorios Newton. Buenos Aires, Argentina).

2.3. Serum samples

The heifers were bled 87 days prior to, and at vaccination and then monthly on 16 occasions during 488 days after vaccination.

The blood was obtained by jugular punction in glass tubes and allowed to clot during 24 hours at room temperature. Then, the serum was separated and stored at -20° C until processed.

2.4. Serologic techniques

2.4.1. Conventional techniques

All the sera were analyzed by RB, BPA, 2ME, SAT and CF. These techniques were performed according to previous descriptions [5-8]. The antigens for all the tests were purchased from the Research Center on Veterinary Sciences, The National Institute of Agricultural Technology (INTA), Castelar, Argentina.

2.4.2. Indirect ELISA (I-ELISA)

This technique was performed using an FAO/IAEA ELISA. The procedures for this technique were published elsewhere [9].

2.4.3. Competitive ELISA (C-ELISA)

This technique was performed according to [10]. Briefly, 96 flat-bottom wells plates (Nunc MicroWell, cat # 2-69620. Denmark) were coated with O-polysaccharide at a concentration of 2 μg/ml. After an overnight period of incubation at room temperature, the plates were washed. Diluted test and control sera (1:50 dilution) were added, to the plates immediately before that a HRP conjugate monoclonal mouse anti O-polysaccharide at a dilution of 1:2500 was added and the plates were incubated for 2 has at room temperature. Then, the plates were washed again and developed using an ABTS/H_2O_2/citrate buffer substrate solution. The reaction was stopped with a 4% sodium dodecyl sulphate solution and the plates were read in a Multiskan Plus ELISA reader using a 405nm filter.

Controls consisted of a buffer control, a negative serum, a vaccinated control on a strong positive reactor. Every control was run in 4 replicates while every test sera was analyzed in duplicate.

The degree of competition was calculated as a proportion of the conjugate control and expressed as percentage inhibition. Values greater than 30% were considered to be positive, while values less than 30% were considered negative. The degree of inhibition was calculated as:

$$\% \text{ inhibition} = 100 - \frac{\text{X OD test serum}}{\text{X OD conjugate control}} \times 100$$

The O-polysaccharide and the anti-O-polysaccharide mouse monoclonal conjugate were provided by the Animal Disease Research Institute, Nepean, Canada.

3. RESULTS

The number and percentage of positive animals to every test before and after vaccination are shown in Table I and Figures 1 to 2. In the two samplings prior vaccination (87 and 0 days before vaccination), 100% of the heifers were negative to all the techniques, while 28 days after vaccination, all the animals were positive reactors to BPA, RB, 2ME, CF, I-ELISA and C-ELISA. The SAT showed agglutinating titers of 1/100 (positive threshold) or higher only in the 71.4% of the animals, wile 28.6% of the heifers showed diverse titers lower than 1/100. This technique gave agglutinating titers at intervals, though titers of 1/100 or higher were observed only until the 148 days after vaccination.

The test that gave positive results for a longest period was BPA that at 419 days after vaccination still detected 6.2% of the animals as positive reactors. At 450 days after vaccination 100% of the heifers were negative to BPA. On the other hand the test that gave positive results for the shortest period was the SAT, which at 148 days after vaccination detected only 6.2 of the heifers as positive reactors, and in the following sampling (190 days post-vaccination) gave negative results in 100% of animals. The 2ME and CF gave 100% of negative results at 235 days after vaccination.

With RB, 100% of the animals were negative at 214 days after vaccination. The I-ELISA technique showed a decreasing percentage of positive animals, with 100% of heifers being negative at 316 days after vaccination. The C-ELISA gave also a decreasing percentage of positive animals, being 100% of heifers negative to this technique at 253 days after vaccination.

TABLE I. NUMBERS AND PERCENTAGES OF POSITIVE ANIMALS TO SEVERAL SEROLOGICAL TESTS BEFORE AND AFTER VACCINATION WITH *BRUCELLA ABORTUS* S-19

Positive to														
	BPA		RB		SAT		2ME		CFT		I-ELISA		C-ELISA	
(*)	+/T	%	+/T	%	+/T	%	+/T	%	+/T	%	+/T	%	+/T	%
(**)		0	0/13	0	0/13	0	0/13	0	0/13	0	0/13	0	0/14	0
0	0/16	0	0/16	0	0/16	0	0/16	0	0/16	0	0/15	0	0/14	0
28	14/14	100	14/14	100	10/14	100	14/14	100	9/9	100	14/14	100	14/15	93
62	16/16	100	8/16	50	3/16	19	13/16	81	3/16	25	13/15	87	5/16	31
90	16/16	100	3/16	19	2/16	12	6/16	37	3/16	19	8/14	57	2/17	12
118	14/15	93	1/15	7	2/15	13	1/15	7	1/14	7	4/15	27	1/14	7
148	14/16	87	1/16	6	1/16	6	1/16	6	1/16	6	2/15	13	1/16	6
190	11/16	69	1/16	6	0/16	0	1/16	6	1/16	6	2/15	13	1/16	6
214	5/16	31	0/16	0	0/16	0	1/16	6	1/16	6	2/16	12	1/15	6
253	4/15	27	0/15	0	0/15	0	0/15	0	0/15	0	1/15	7	0/15	0
289	4/15	27	0/15	0	0/15	0	0/15	0	0/15	0	1/15	7	0/9	0
316	4/16	25	0/16	0	0/16	0	0/16	0	0/16	0	0/15	0	0/16	0
328	2/16	0	0/16	0	0/16	0	0/16	0	0/16	0	0/16	0	0/11	0
363	1/16	0	0/16	0	0/16	0	0/16	0	0/16	0	0/13	0	0/16	0
393	1/15	0	0/15	0	0/15	0	0/15	0	0/15	0	0/14	0	0/8	0
419	1/16	6	0/16	0	0/16	0	0/16	0	0/16	0	0/16	0	0/16	0
450	0/16	0	0/16	0	0/16	0	0/16	0	0/16	0	0/16	0	0/9	0
488	0/16	0	0/16	0	0/16	0	0/16	0	0/16	0	0/16	0	0/15	0

(*) Days after vaccination
(**) Sampling 87 days after vaccination
+/T number of positive reactors/number of animals tested
% percentage of positive animals

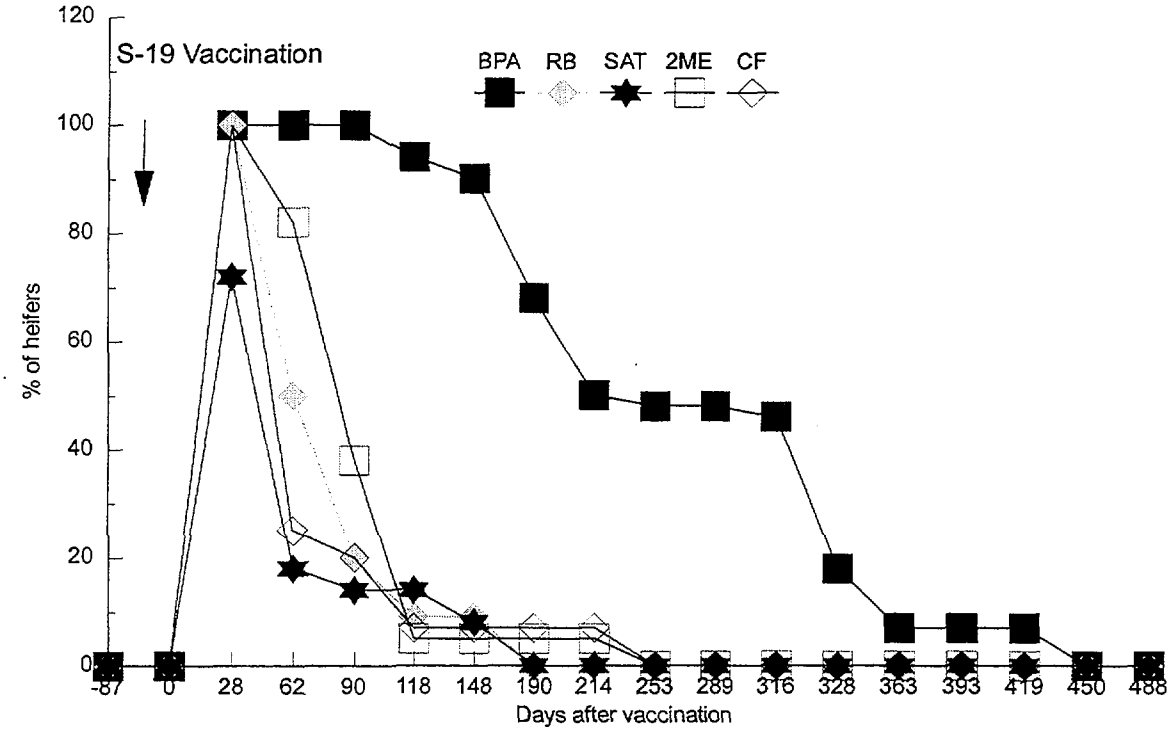

FIG. 1. Percentage of heifers positive to different conventional tests after S-19 vaccination.

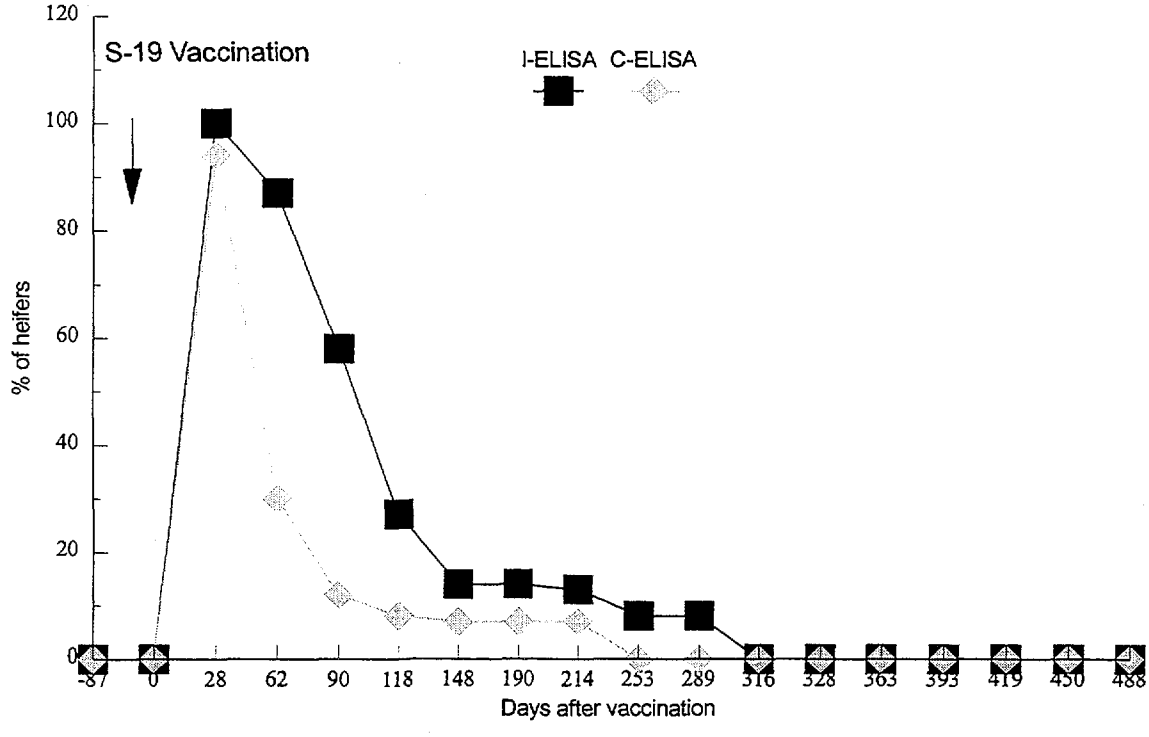

FIG. 2. Percentage of heifers positive to I-ELISA and C-ELISA after S-19 vaccination.

4. DISCUSSION

We evaluated the humoral immune response to eight serologic techniques of 16 heifers vaccinated with standard dose of B. abortus S-19. The S-19 is a live, attenuated vaccine that protects cattle against infection with B. abortus. However, since the 1950s it is known that vaccination of cattle with S-19 produces persistent antibodies that make difficult the interpretation of diagnostic serological test [3]. It has also been shown proved that the younger the heifers are vaccinated, the less persistent are the antibodies [4, 10, 11, 17].

Sutherland [12], vaccinated 24 heifers between 3 and 6 months with standard dose of strain 19 and evaluated the sera at 2 and 3 months after vaccination by RB, SAT, CF and I-ELISA. The RB detected as positive reactors 100% of the animals until 3 months after vaccination, which differs from the results showed here that indicated that at 3 months after vaccination only 19% of the animals were positive to RB. In the study referred to, the positivity to SAT descended from 100% to 50% between the second and third month, and the CF descended from 87% to 37% in the same period, while the I-ELISA remained positive in 87% for the two both months. In the present study, excluding the results of I-ELISA, which agreed with those of Sutherland (1984/85) at 2 months post-vaccination, all the other values were lower than those found by that author.

Cunningham and O'Reilly [4] vaccinated heifers from 3 to 6 months of age with strain 19. Sera were analyzed weekly by SAT for 8 months. They obtained titers lower than 1/100 prior to 2 months post-vaccination which indicates that the decline in antibody of all the animals occurred earlier than in our study. Casaro [13] vaccinated heifers between 5 and 7 months of age with S-19 and measured antibodies until 362 days post-vaccination by SAT and RB. He found that 100% of the animals were negative at 105 days with SAT and at 158 days with RB. These results differ moderately from those obtained in our assay (100% negative to SAT and to RB at 190 days and at 214 days after vaccination, respectively). Nagy et al. [3] reported that 100% of the animals were negative to SAT at 180 days after vaccination in heifers vaccinated between 4 and 8 months of age with strain 19. Those results agree with ours.

A possible explanation for the difference in results between different authors could be the use of different vaccines, doses and age in the immunization of the animal and the use of different antigens and other reagents. Finally, a different immunologic status of the animals can not be ruled out.

Traditionally, the CF has been used as a definitive test of high sensitivity and specificity for reactors in agglutination tests [12, 14]. In the present study, the CF did not detect any positive reactors at 253 days after vaccination (approximately 13.5 months of age) which provides a broad margin of security in the serological testing at 18 months of age. However, in spite of its great sensitivity and specificity, this is a cumbersome, time consuming technique which is also difficult to standardize.

The ELISA, on the other hand, is free from all the problems of the agglutination and CF technique. This technique is highly sensitive and specific and detects all the isotypes of IgG and IgM present in serum [15]. In addition, it is rapid and requires a minimum amount of serum.

In our study, the I-ELISA detected 100% of the animals positive at 28 days after vaccination, decreasing this percentage until the 148 days after vaccination when only 13.3% of the animals were positive, 100 % of the heifers being negative at 316 days after vaccination (approximately 15.5 month of age). The C-ELISA here used was developed to differentiate vaccinated from infected cattle [10]. In our study, we found positive animals to C-ELISA until 214 days after vaccination. Nielsen et al [16], found that antibodies produced to B. abortus strain 19 cannot compete in the C-ELISA, with two major exceptions: animals persistently infected with B. abortus, and occasionally, antibody at the peak of the primary anti-strain 19 antibody response 4-8 weeks post vaccination, the latter competition being weak at best. In our case, persistent infection with S-19 can be ruled out as at 450 days after vaccination, all the heifers became negative in all the techniques. The difference in duration of antibodies found in our study with that stated by Nielsen et al [16] could, at least partially, be explained by the different doses of S-19 used in different countries. The C-ELISA improved the result, of the I-ELISA as at 253 days after vaccination it did not detect any positive reactor. These results make the I-ELISA and C-ELISA useful for diagnosis of bovine brucellosis, even according to the new Argentine regulations that establish compulsory serological testing from the 18 month of age (Resolución 1269/93 del Servicio Nacional de Sanidad Animal, 16-11-93).

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