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CROSS-REACTIONS BETWEEN *Yersinia enterocolitica* SEROTYPE 9 AND *Brucella* spp IN BOVINE AND SWINE SERA, IN THE AREA OF RIO DE JANEIRO[1]

Norma S. Lázaro[2] e Ernesto Hofer[3]

SINOPSE.- Lázaro N.S. & Hofer E. 1996. [**Reações cruzadas entre Yersinia enterocolitica sorotipo 9 e Brucella spp em soros de bovinos e suínos, na área do Rio de Janeiro.**] Cross-reactions between Yersinia enterocolitica serotype 9 and Brucella spp in bovine and swine sera, in the area of Rio de Janeiro. *Pesquisa Veterinária Brasileira 16(1):39-43.* Depto Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ 23851-970, Brazil.

O grau de interferência antigênica entre *Yersinia enterocolitica* O:9 e *Brucella* spp foi analisado em 245 soros de bovinos discriminados quanto ao estado de imunização contra brucelose, e 119 soros de suínos. Os espécimens foram submetidos às provas de soro-aglutinação rápida, soro-aglutinação lenta e antígeno acidificado para *Brucella* spp e soro-aglutinação lenta para *Y. enterocolitica* O:9. A resposta imune a *Y. enterocolitica* foi evidenciada e considerada significativa em relação aos seus possíveis efeitos na interpretação dos testes sorológicos para brucelose. Os títulos aglutinantes foram aproximados ou mesmo similares do ponto de vista quantitativo. Como não foi possível determinar com precisão, na maioria dos casos, o agente etiológico, é necessário instituir um método laboratorial de diagnóstico de brucelose que permita distinguir as infecções específicas.

TERMOS DE INDEXAÇÃO: *Yersinia enterocolitica, Brucella* spp, reação cruzada, bovino, suíno.

ABSTRACT.- The extent of antigen interference between *Yersinia enterocolitica* O:9 and *Brucella* spp was evaluated in 245 bovine serum samples divided into groups according to status of immunization against brucellosis, and in 119 swine serum samples. The specimens were submitted to the plate serum agglutination test, tube serum agglutination test and to the Rose Bengal test for *Brucella* spp, and to tube serum agglutination for *Y. enterocolitica* O:9. The immune response to *Y. enterocolitica* was demonstrated and considered significant in terms of its possible effects on the interpretation of serological tests for brucellosis. Agglutinating titers were close or even similar from a quantitative viewpoint. Since in most cases it was not possible to determine precisely the etiologic agent, it is clearly necessary to set up a laboratory method for the diagnosis of brucellosis that will permit the distinction of specific infections.

INDEX TERMS: Brucellosis, Yersiniosis, cross-reaction, *Yersinia enterocolitica* O:9, *Brucella* spp.

INTRODUCTION

Yersinia 3enterocolitica O:9 is a microorganism that paradoxically is of importance in Veterinary Medicine not because of its occurrence in animals, but because of its antigenic relationship with *Brucella* (Ahvonen et al. 1969). The two microorganisms share a somatic antigen, so that the immune response of animals exposed to *Y. enterocolitica* 0:9 will be difficult to distinguish from that induced by *Brucella abortus* (Corbel & Cullen 1970, Hurvell 1973). This fact implies the elimination of animals with false-positive results, especially in countries in wich measures for the eradication of brucellosis are based on serological tests.

In view of the marked prevalence of brucellosis among Brazilian herds, the objective of the present study was to evaluate the extent of antigenic interference between *Y. enterocolitica* O:9 and *Brucella* spp in cattle and swine using the tecniques routinely employed for the serological diagnosis of brucellosis and yersiniosis.

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[2] Depto Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, RJ 23851-970, Brazil.

[3] Depto Bacteriologia, FIOCRUZ, Rio de Janeiro, RJ 21045-900, Brazil.

MATERIALS AND METHODS

Sampling

A number of 245 serum samples from cattle and 119 from swine raised in various regions of the State of Rio de Janeiro, Brazil, were analyzed. An important aspect of the study was to characterize and distinguish samples from animals that had been immunized or not against brucellosis. Thus, 185 serum samples from non-vaccinated cattle of different age ranges and 60 from vaccinated cattle at the age of ≥ 30 months were studied.

The sera were inactivated at 56°C for 30 minutes on a water bath stored at -20°C until the time for use.

Antigens

Brucella abortus - for the plate and tube agglutination test antigens were prepared and standardized at the Zoonoses Laboratory of the Department of Microbiology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, according to the technique recommended by WHO (Alton et al. 1977). In addition to the two classical tests, acidified and Rose Bengal-stained antigen was used, sold by Becton, Dickinson and Company (USA) under the generic name of Card Test (CT).

Yersinia enterocolitica O:9 - strain M.Y. 79 (biotype 3, serotype 9, undetermined phagotype), were used, kindly provided by Prof. S. Winblad, Institute of Clinical Bacteriology, Malmoe, Sweden. Somatic antigen was obtained by the technique recommended by Hofer (see Lázaro 1980).

Serological tests

Serum agglutination for *Brucella* spp - the plate (rapid) and tube (slow) serum agglutination techniques (PSA and TSA) were carried out and interpreted according to the recommendations of Alton et al. (1977). The Card Test was performed as recommended by Nicoletti (1967).

In the rapid and slow tests, agglutinating titers ≥ 100 and ≥ 80 were considered to be positive for non-vaccinated cattle, and agglutinating titers ≥ 200 and ≥ 160 were considered to be positive for vaccineted animals. The positivity threshold for swine was ≥ 50 and ≥ 40 for the two tests, respectively. In the Card Test, only the presence or absence of agglutination was considered.

The TSA for *Y. enterocolitica* O:9 was performed as done for *Brucella* spp, with the following modifications: the serum-antigen system was incubated for two hours at 50°C (water bath) and further incubated for 22 hours at 37°C. Reactions with titers ≥ 80 were considered to be positive.

RESULTS

The data in Table 1 show that the results of the TSA tests for *Brucella* sp and *Yersinia enterocolitica* O:9 (38 sera) were analogous, whereas in vaccinated animals, slow serum agglutination and CT presented similar results (4 positive sera). In swine, the PAS (35 sera), CT (30 sera) and TSA tests for *Y. enterocolitica* O:9 (32 positive sera) were characterized by homogeneous results, with the largest number of *Brucella*-positive animals being detected with TSA (59 sera).

In the different associations investigated with respect to the tests for *Brucella* and *Y. enterocolitica* O:9, a predominance of sera reacting to *Y. enterocolitica* O:9 was observed in vaccinated and non-vaccinated cattle. In these associations, the nonparametric chisquare test showed a significant difference at the 1% level of probability ($p < 0.01$). In this case, we assumed a predominance of infection with *Y. enterocolitica* O:9, even though antigenic interference between *Brucella* sp. and *Y. enterocolitica* was demonstrated.

With respect to the TSA test for non-vaccinated cattle and the PSA and CT tests for swine, no significant difference in pattern was observed, i.e., there was no prevalence of a given agent over the other.

The TSA test for *Brucella* in swine sera showed a significant difference for *Brucella* sp, with no serum reacting only to *Y. enterocolitica* O:9.

Finally, Table 3 shows that in the positivity pattern revealed by the four tests, swine presented a much higher percentage than did cattle. However, in contrast to cattle, no swine serum presented an individual reaction to *Y. enterocolitica* O:9, but reacting sera were always positive to both *Y. enterocolitica* and *Brucella* spp.

In the overall analysis, considering the 129 sera that reacted with any one of the antigens employed, 60 showed cross-reactions between *Brucella* and *Y. enterocolitica* O:9. It shoud also be pointed out that 46 sera presented positive reactions with one of the *Brucella* antigens and 23 others revealed specificity for *Y. enterocolitica* O:9.

DISCUSSION

A careful analysis of Table 1 shows that in both cattle classes (vaccinated and non-vaccinated animals) the CT showed higher percentages of positive reactions than PSA. This was mainly due to the reduction of titers in PSA with the inclusion of suspected animals (1:50 and 1:100, respectively) in the negative group. However, among the samples tested by CT, some reacted positively as a function of the immunoglobulin class involved since, according to Morgan et al. (1969) and Levieux (1974), IgG's play a fundamental role in the Rose Bengal test.

Another interest point regarding vaccinated animals was the low rate of sera reacting to *Brucella*, in contrast to the higher percentage of animals that reacted to *Yersinia enterocolitica* O:9. In this case, one cannot state that these animals were infected by *Y. enterocolitica* since this result very probably was due to animals with 1:200 I (incomplete) titer in PSA and 1:160 I in TSA, which were considered suspected according to the criterion of interpretation for vaccinated animals and that in the present study were included in the negative class.

On the other hand, when the results for the 60 sera from vaccinated cattle are considered individually, it can be seen that among those positive to *Y. enterocolitica* O:9 one animal was positive by CT but showed very low titers in the PSA and TSA tests (1:25 and 1:40, respectively). We may propose that the animal was previously infected with *Y. enterocolitica* O:9, although the natural occurrence of this microorganism in cattle is relatively rare (Gueraud et al. 1995, Hilbink et al. 1995). Conversely, there are many reports of data obtained by experimental infection that agree with the above problem (Corbel & Cullen 1970, Mittal et al. 1980, Mathias et al. 1988).

Table 1. Frequency of sera from cattle vaccinated or not against brucellosis and from swine that reacted positively to *Brucella* spp and *Yersinia enterocolitica* serotype 9

Tests	Cattle				Swine	
	Non-vaccinated		Vaccinated		React. (%)	Pos. (%)
	React. (%)	Pos. (%)	React. (%)	Pos. (%)		
PSA	52 (28.1)	10 (5.4)	06 (10.0)	01 (1.7)	46 (38.6)	35 (29.4)
TSA	127 (68.6)	38 (20.5)	27 (45.0)	04 (6.7)	71 (59.7)	59 (49.6)
Card Test	-	20 (10.8)	-	04 (6.7)	-	30 (25.2)
Y. enterocolitica 0:9	104 (56,2)	38 (20.5)	36 (60.0)	13 (21.7)	73 (61.3)	32 (26.9)

React. = Reactor
Pos. = Positive
PSA (Plate serum agglutination) PSA = ≥ 1:25 Cattle non-vaccinated ≥1:100 TSA ≥ 1:20 Cattle vaccinated ≥ 1:200 Swine ≥ 1:50
TSA/Ba/Y.e.9 (Tube serum agglutination) ≥ 1:80 ≥ 1:160 ≥ 1:40

Table 2. Behavior of cattle and swine sera in the presence of *Brucella abortus* and *Yersinia enterocolitica* O:9 antigens

Animals	*Y. enterocolitica* 0:9 reaction	*Brucella* spp					
		PSA		TSA		CT	
		+	-	+	-	+	-
Cattle non-vaccinated	-	3	144	17	130	2	145
	+	7	31	21	17	18	20
X^2		23.05[a]		0[n.s.]		14.08	
Cattle vaccinated	-	-	47	-	47	-	47
	+	1	12	4	9	4	9
X^2		12		9		9	
Swine	-	10	77	27	60	5	82
	+	25	07	32	0	25	7
X^2		0.53[n.s.]		27.0		0.33[n.s.]	

[a] At the 1% level of probability ($p<0.01$).
PSA - Plate serum agglutination test.
TSA - Tube serum agglutination test.
CT - Card Test.

Among the anomalous reactions was that of a vaccinated cow with a 1:160 titer in TSA and negative result in CT, and another with 1:40 titer in TSA but positive to CT and revealing titers of 1:1280 ad 1:80, respectively, in relation to *Y. enterocolitica* O:9.

Table 2 shows that of the sera from 41 non-vaccinated cows that reacted with one of the antigens, 31 exclusively reacted to *Y. enterocolitica* O:9. Perhaps an explanation for this occurrence was the negative titer of animals with suspected reaction in the PSA test. With respect to the vaccinated cattle, only one of the 13 sera reacting to *Y. enterocolitica* was also positive for *Brucella* in the PSA test.

Analysis of the results for the 119 swine in the PSA and CT shows that 25 sera were positive for both microorganisms, whereas a relatively small number reacted separately to *Brucella* and/or *Y. enterocolitica* O:9. The CT was positive in almost all samples when PSA or TSA, or both, were positive. Nicoletti (1967) stated that the Card Test is the most efficient process for the screening of infected swine when all the members of a herd are analyzed.

The TSA test, in addition to demonstrating antigenic interference, showed the predominance of *Brucella* infection (59 pigs) since no animal reacted only to *Y. enterocolitica* O:9.

The above data suggest that the combination of TSA for *Brucella* with TSA for *Y. enterocolitica* for non-vaccinated cattle does not distinguish between animals infected with only one of the two agents. Despite the evidence for antigenic interference, the possibility of greater involvement by *Y. enterocolitica* O:9 was clear for vaccineted cows, whereas infection with *Brucella* spp predominated among swine.

The data for non-vaccinated cattle show that the combination of CT with serum agglutination for *Y. enterocolitica* characterized the predominance of *Yersinia* without

Table 3. Distribution and frequency of the reactive patterns of positive cattle and swine sera in the presence of *Brucella* spp (PSA-TSA-Card Test) and *Yersinia enterocolitica* serotype 9 antigens

Techniques[a]				Animals							
				Cattle							
				Non-vaccinated		Vaccinated		Swine		Total	
PSA	TSA	CT	Y.e.9	No.	%	No.	%	No.	%	No.	%
-	-	-	-	128	69.18	47	78.33	60	50.42	235	64.56
+	+	+	+	7	3.78	1	1.66	24	20.16	32	8.79
-	+	-	-	15	8.10	-	-	16	13.44	31	8.51
-	-	-	+	15	8.10	8	13.33	-	-	23	6.31
-	+	+	+	9	4.86	2	3.33	1	0.84	12	3.29
-	+	-	+	5	2.70	1	1.66	6	5.04	12	3.29
+	+	-	-	1	0.54	-	-	6	5.04	7	1.92
-	-	+	+	2	1.08	1	1.7	-	-	3	0.82
+	-	-	-	1	0.54	-	-	-	-	1	0.27
-	-	+	-	1	0.54	-	-	-	-	1	0.27
-	+	+	-	-	-	-	-	1	0.84	1	0.27
-	+	+	-	1	0.54	-	-	4	3.33	5	1.37
+	+	-	+	-	-	-	-	1	0.84	1	0.27
Total				185	99.96	60	99.98	119	99.94	364	99.94

[a] As indicated in Table 1.

masking the antigenic interference of *Brucella*. These results were also observed for vaccinated cattle, with a higher frequency of animals reacting to TSA, CT and *Y. enterocolitica* O:9 compared to PSA. In the analysis of this aspect, it is important to consider the participation of the evolutionary stage of the infection and/or disease, basically considering that TSA has limitations during the incubation and chronic phases of the disease, and also in the presence of vaccination (Morgan et al. 1969) and that the Card Test can recognize reacting animals before they are detected by TSA, although it does not discriminate between infected animals and animals immunized with agglutinogenic vaccine (Nicoletti 1967, Morgan et al. 1969, Pilet et al. 1972).

In the present study, in view of the age when the animals were tested, positive reactions to the different tests possibly originated from later infection with *Brucella* or *Y. enterocolitica* O:9 and not due to agglutinins of the vaccine. It should be pointed out that a vaccinated cow with a 1:80 titer in SSA, and therefore positive for brucellosis, presented the same titer for *Y. enterocolitica* O:9 and was then defined as positive, although this reaction was attributed to cross-antigenicity. However, we obtained sera with 1:320 and 1:160 titers for *Y. enterocolitica* O:9, which reacted at 1:20 and 1:40 in TSA, respectively, and did not react in PSA, indicating a probable infection with *Y. enterocolitica* O:9. We also observed that three sera that showed 1:80 titers for *Y. enterocolitica* O:9 did not react in the other tests.

On the basis of these results, one may admit that under natural conditions the animals infected with *Yersinia enterocolitica* O:9 may react or not to *Brucella* depending on the evolutionary phase of the infection.

Corbel (1973) pointed out that the antibodies that cross-react in the serum of cattle inoculated with *Yersinia enterocolitica* O:9 are qualitatively similar to those of *Brucella* spp but become differentiated in chronological order, undergoing a rapid decline.

Several techniques for the distinction between the infection caused by the two etiological agents have been investigated. Corbel & Cullen (1970) reported that cattle and rabbits immunized with *B. abortus* and *Y. enterocolitica* present a cross-reaction in the Rose Bengal plate tests and that only a quantitative test using suspensions of *B. abortus* and *Y. enterocolitica* O:9 stained with Rose Bengal permitted the specification of the immunologic action of the two bacteria.

By inoculating cattle with *Y. enterocolitica* O:9, Mittal & Tizard (1979, 1980) demonstrated that homologous titers were consistently higher than heterologous titers in the microplate serum agglutination test using tetrazolium-stained antigens.

Das & Paranjape (1987), in a study using the quantitative plate test with a somatic (O) and flagellar (H) somatic antigen of *Y. enterocolitica* O:9, stabilized and stained with Rose Bengal, reported that antigen H of *Y. enterocolitica* O:9 was the only process capable of differentiating between the serological responses of the two microorganisms. Similarly, Mathias et al. (1987) reported that the determination of agglutinating and complement-fixing antibodies against the somatic antigens of the two bacteria and against the flagellar antigen of *Y. enterocolitica* O:9 permits the differentiation between brucellosis and yersiniosis in experimentally infected animals.

Finally, the multiple reaction profiles presented in Table 3 show that swine were the animals that presented the highest rate of sensitization by *Brucella* sp, followed by unvaccinated cattle. Vaccinated cattle did not react to any

of the *Brucella* tests except when the tests were combined with those for *Y. enterocolitica*. Reactions to low titers were observed, but did not reach positivity. These reactions probably originated from vaccination.

In terms of the response to the *Y. enterocolitica* O:9 antigen only, vaccinated cattle showed the highest sensitization (13.3%), followed by non-vaccinated cattle (8.10%), whereas swine did not react separately but presented agglutinins only in the tests combined with those for *Brucella* spp.

In summary, CT and PSA gave more conclusive results for a specific diagnosis in swine, whereas PSA detected fewer cross-reacting animals among cattle.

The immune response to *Y. enterocolitica* O:9 was demonstrated and considered to be significant with respect to its possible effects on the interpretation of serological tests for brucellosis. The agglutinating titers were close or even similar from a quantitative viewpoint and in most cases the etiologic agent could not be precisely determined. A laboratory method for the diagnosis of brucellosis that will permit to distinguish between specific infections is definitely needed.

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REFERENCES

Ahvonen P., Janson E. & Aho K. 1969. Marked cross agglutination between *Brucellae* and a subtype of *Yersinia enterocolitica*. Acta Pathol. Microbiol. Scand. 75:291-295.

Alton G.G., Jones L.M. & Pietz D.C. 1977. La brucellose techniques de laboratoire. 2ième éd. Genéve. Organization Mondiale de la Santé (Ser. Mon. no. 55), p. 68-76.

Corbel M.G. 1973. The nature of the antibody response to *Yersinia enterocolitica* in cattle. J. Hyg. 71:309-323.

Corbel M.J. & Cullen G.A. 1970. Differentiation of the serological response to *Yersinia enterocolitica* and *Brucella abortus* in cattle. J. Hyg. 68:519-530.

Das A.M. & Paranjape V.L. 1987. A stabilized Rose Bengal antigen plate test for differentiation of serological response evoked by *Yersinia enterocolitica* serotype O:9 and *Brucella abortus* in bovine sera. Indian J. Anim. Sci. 57: 633-638.

Gueraund J.M., Garin-Bastuji B., Cau C., Connetable N., Jourdain D., Laubier S., Sarton C. & Thiebaud M. 1995. Isolement de *Yersinia enterocolitica* O:9 à partir de prélèvements d'avortement chez une vache présentant une réaction sérologique positive en brucellose. Bull. Acad. Vét. de France 68: 75-80.

Hilbink F., Fenwick S.G., Thompson E.J., Kittelberger R., Penrose M. & Ross G.P. 1995. Non-specific seroreactions against *Brucella abortus* in ruminants in New Zealand and the presence of *Yersinia enterocolitica* O:9. N. Z. Vet. J. 43: 175-178.

Hurvell B. 1973. Serological cross-reaction between different *Brucella* species and *Yersinia enterocolitica* agglutinating activity of 19S and 7S antibodies against somatic antigen of *Brucella abortus* and *Yersinia enterocolitica* type IX. Acta Vet. Scand. 14: 474-488.

Lázaro N.S. 1980. Reações sorológicas cruzadas entre *Yersinia enterocolitica* sorotipo 9 e *Brucella* sp em bovinos e suínos. Tese de Mestrado, UFMG, Belo Horizonte. 72p.

Levieux D. 1974. Bovine immunoglobulins and brucellosis I:II. Ann. Rech. Vét. 5: 332-353.

Mathias L.A., Pinto A.A., Girio R.J.S. & Irino K. 1987. Diferenciação entre infecção experimental por *Brucella abortus* e por *Yersinia enterocolitica* sorogrupo 09 em cobaias, através das provas de soroaglutinação e de fixação de complemento. Ars Vet. 3: 55-62.

Mathias L.A., Pinto, A.A., Girio, R.J.S. & Perecin, D. 1988. Use of complement fixation test in the differentiation of *Brucella abortus* and *Yersinia enterocolitica* serogroup 09 experimental infection in bovine. Revta. Microbiol. 19: 148-154.

Mittal K.R. & Tizard I.R. 1979. Experimental studies on the saerologic relationships between *Yersinia enterocolitica* and *Brucella abortus*. Res. Vet. Sci. 27: 354-360.

Mittal K.R. & Tizard I.R. 1980. The microplate agglutinate test. A simple technique to assist in the differentiation of bovine brucellosis and Yersiniosis. Vet. Rec. 106: 403-405.

Mittal K.R., Barnum D.A. & Tizard I.R. 1980. Experimental infection of cattle with *Yersinia enterocolitica* serotype 09: serologic responses. Am. J. Vet. Res. 41: 1607-1610.

Morgan W.J.B., Mackinnon D.J., Lawson J.R. & Cullen G.A. 1969. The Rose Bengal Plate Agglutination Test in the diagnosis of brucellosis. Vet. Rec. 85: 636-641.

Nicoletti P. 1967. Utilization of the card test in brucellosis erradication. J. Am. Vet. Med. Assoc. 151: 1778-1783.

Pilet C.H., Toma B. & André G. 1972. Diagnostic sérologique de la brucellose par l'Épreuve a l'Antigéne Tamponné (EAT) ou Card Test. Cah. Méd. Vét. 41: 5-19.