

ANTI-*Yersinia enterocolitica* SEROTYPE 3 AGGLUTININS IN SWINE SERA FROM RIO DE JANEIRO¹

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ABSTRACT.- Lázaro N.S. & Hofer E. 1997. Anti-*Yersinia enterocolitica* serotype 3 agglutinins in swine sera from Rio de Janeiro. *Pesquisa Veterinária Brasileira* 17(1):9-11. Depto Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ 23851-970, Brazil.

The slow serum agglutination test was applied to 119 healthy pigs for the determination of the possible presence of anti-*Yersinia enterocolitica* O:3 agglutinins. Of the 63.9% reactive animals ($\geq 1:20$), 8.4% presented positive titers ($\geq 1:80$), suggesting the presence of this pathogen among swine and consequently an additional public health problem.

INDEX TERMS: *Yersinia enterocolitica*, agglutinins, swine.

SINOPSE.- Aglutininas anti-*Yersinia enterocolitica* O:3 em soros de suínos do Rio de Janeiro. A pesquisa de aglutininas anti-*Yersinia enterocolitica* O:3 foi realizada em 119 suínos saudáveis, através da prova de soro-aglutinação lenta. Dos 63,9% animais reagentes ($\geq 1:20$), 8,4% apresentaram títulos a nível de positivo ($\geq 1:80$) sugerindo a presença deste patógeno em nossos rebanhos suínos constituindo-se consequentemente em mais um problema de saúde pública.

TERMOS DE INDEXAÇÃO: *Yersinia enterocolitica*, aglutininas, suíno.

INTRODUCTION

Infection with *Yersinia enterocolitica* causes several clinical problems in swine, with aggression mainly at the enteric level, although a less frequent occurrence of extraintestinal infection has also been reported (Ahvonen et al. 1973). An important feature observed in swine is the marked occurrence of asymptomatic carriers (Mollaret et al. 1979, Hurvell 1981, Adesiyun et al. 1992), with significant isolation of *Y. enterocolitica* in some European countries (Pedersen 1976, Wuthe et al. 1982), Japan (Zen-Yoji 1981), Canada (Schiemann & Fleming 1981) and Brazil (Falcão 1981, Castro et al. 1983, Lázaro et al. 1986, Mendonça et al. 1992, 1995). The strains isolated from swine usually belong to the serotypes

responsible for human enteritis, O:3 in particular. In view of the lack of reports on this subject in the Brazilian literature, and on the basis of the notifications of isolation of this microorganism in our country, the objective of the present study was to determine the frequency of *Y. enterocolitica* O:3 dissemination among Brazilian swine by searching for circulating agglutinins through the slow serum agglutination test.

MATERIALS AND METHODS

Sampling

We used 119 healthy pigs of different age ranges originating from twelve properties in the municipal districts of Itaguaí, Seropédica and Santa Cruz, in the state of Rio de Janeiro, Brazil. The meat was produced for domestic consumption and for sale on the street. Blood samples (5 ml) were obtained by puncture of the marginal vein of the ear and sera were inactivated at 56°C for 30 minutes in a water bath and stored at -20°C until the time for use.

Y. enterocolitica antigen

The strain used was M.y1, biotype 4, serotype 3 and phagotype VIII from the M.Y. (Malmo *Yersinia enterocolitica*) collection, kindly provided by Prof. S. Winblad, Institute of Clinical Bacteriology, Malmo, Sweden.

Antigen was obtained by the method of Hofer (see Lázaro 1980). The strain was cultured on Agar Mueller-Hinton medium (DIFCO) and incubated at 25°C for 24 to 48 hours. The growth suspension was obtained with saline containing 0.2 g% 2-3-5 triphenyltetrazolium chloride (TTC-Difco) and incubated at 37°C for 4 to 6 hours, the time needed for TTC

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reduction to formazan (demonstrated by the development of red color). The suspension was then washed three times in saline by centrifugation at 3000 rpm for 20 minutes. Ten ml of a solution of 1 ml glycerol p.a., 1 ml liquid phenol p.a. and 98 ml 0.9% saline sterilized at 121°C for 30 minutes were added to the colored deposit. At the time of use, the preparation was diluted in 0.3 g% phenol-saline so as to obtain turbidity corresponding to tube 2 of the nephelometer of McFarland (6×10^8 bacteria/ml). The original suspension is stable for up to 3 months when stored at 4-8°C.

Slow serum agglutination test

A few assays were first performed in order to obtain a sufficiently stable standardized technique for the detection of anti-*Yersinia enterocolitica* O:3 agglutinins, with special emphasis on parameters such as temperature and time of exposure to the antigen-antibody pair. Specific somatic antisera obtained from rabbits immunized with standard strains were used, with titers ranging from 1:640 to 1:2,560.

In the tube agglutination test, swine sera were diluted and supplemented with antigens at final titers of 1:20, 1:40, 1:80 and so forth. The material was incubated in a water bath for 2 hours at 50°C and then for 22 hours at 37°C.

The choice of an agglutinating titer to be considered as a reference point characterizing the possible states of infection/disease in swine was based on the observations of Winblad (1968), Ahvonen & Sievers (1969) and Wauters (1970), who adopted a titer of 1:80 as the initial mark of positivity for the serologic diagnosis in humans.

RESULTS AND DISCUSSION

The results presented in Table 1 show that 76 (63.9%) of the 119 pigs analyzed were sensitized to *Y. enterocolitica*, with titers $\geq 1:20$, although only 20 (8.4%) were positive, presenting titers $\geq 1:80$ (Table 2).

Analysis of the individual behavior of each serum sample in the presence of the *Yersinia enterocolitica* O:3 antigen showed that some animals reacted with high titers and could therefore be considered to be in a state of infection and/or disease due to this serotype, or, to a lesser extent, due to a serotype related to it. It should be pointed out that, even though the prevalence of seropositive animals was low, this result confirms literature

Table 1. Frequency of seroreacting ($\geq 1:20$) to *Yersinia enterocolitica* serotype 3 of the 119 swine

Titers	Seroreacting	
	n	%
20	38	31.9
40	28	23.5
80	05	4.2
160	02	1.7
320	03	2.5
Total	76	63.9

n = number of swine.

Table 2. Frequency of seropositive ($\geq 1:80$) to *Yersinia enterocolitica* serotype 3 of the 119 swine

Titers	Seropositive	
	n	%
80	05	4.2
160	02	1.7
320	03	2.5
Total	10	8.4

n = number of swine.

reports that swine play a relevant role in the epidemiology of infection with this serotype (Pedersen 1976, Nesbakken & Kapperud 1985). Indeed, a strong correlation has been demonstrated between the presence of *Y. enterocolitica* O:3 in man and in swine sharing the same geographic area (Christensen 1980, Schiemann & Fleming 1981).

By comparing the frequency of seroreactive (63.9%) and positive (8.4%) animals observed in the present study with the isolation of *Y. enterocolitica* from swine reported by others, we observe that Nesbakken (1985, 1988) obtained 68.1% and 83.3% positivity, respectively, Hariharam et al (1995) obtained 42.0% isolation of *Y. enterocolitica* serotype O:3, although values of less than 6% have been reported by Hunter et al. (1983) and Okoroafor et al. (1988).

Data about the isolation of *Y. enterocolitica* O:3 from swine in Brazil are scarce. Lázaro et al. (1986) isolated *Y. enterocolitica* serotype 3, biotype 4, lysotype VIII from the feces of swine with diarrhea, and Mendonça et al. (1992) obtained a 50.0% rate of carrier swine in Rio de Janeiro.

With respect to the serologic diagnosis of infection with *Y. enterocolitica*, Falcão et al. (1979) analyzed 1554 sera from swine from the states of São Paulo, Parana and Santa Catarina for the presence of *Y. enterocolitica* serotypes O:3, O:5 and O:9 and detected 31.3% sera reacting to one or more serotypes. Lopes & Falcão (1980), in a study of 1,609 human sera for the presence of *Y. enterocolitica* O:3, obtained 0.62% positivity, and Nattermann et al. (1986) obtained 1.4% seropositivity in a study of 3,534 swine.

According to Graux & Wauters (1966), serodiagnosis is a highly valuable process for a late or retrospective diagnosis in familiar or epidemic cases. In the present study, we noted that the high percentage (63.9%) of reacting swine (titer $\geq 1:20$) was possibly due to the fact that these animals, at some time in their life, had been sensitized to *Y. enterocolitica* O:3 or even to another closely related serotype, as deduced from the large number of sera reacting at low titers.

In this respect, Winblad & Sternby (1966) pointed out that anti-*Y. enterocolitica* antibodies are detectable only one week after the clinical manifestations, and Schiemann (1988) did not observe a correlation between severity of the infection and antibody level in the serum of experimentally inoculated swine.

Although in the present study the frequency of seropositive animals was low (8.4%), the results confirm the reports in the Brazilian literature showing that this pathogen circulates in

our midst (Castro et al. 1983, Lázaro et al. 1986, Mendonça et al. 1992).

In addition, serum diagnosis may be useful as an auxiliary method in the control of this disease on rearing farms and at abattoirs and meat packing plants where swine, as well as the food obtained from them, represent an important source of infection for man.

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