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Isolation of *Yersinia pseudotuberculosis* from buffalo (*Bubalus bubalis*) feces

Isolamento de *Yersinia pseudotuberculosis* de fezes de búfalo (*Bubalus bubalis*)

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SUMMARY

Fecal samples from 119 healthy buffaloes, from 5 farms, diluted in 10% brain heart infusion, were maintained for 3 weeks at 4°C and subcultured weekly onto Mac Conckey agar and *Yersinia* selective agar with antimicrobial supplement. *Yersinia pseudotuberculosis* serotype OIII was isolated from only one farm, where an outbreak of yersiniosis was occurring. The bacteria was isolated in 21 of the 25 samples from adult, healthy females and in all the 9 samples from healthy calves. It was not isolated in the samples from other farms, 2 especially, where yersiniosis had been diagnosed 1 and 5 years before. Of the 30 isolates, 14 (46.7%) were recovered from both culture media, one (3.3%) only in Mac Conckey and 15 (50%) only in *Yersinia* selective agar. Of the 15 isolates recovered in Mac Conckey, 12 (80%) were isolated after 1 week of cold enrichment, 3 (20%) after 2 weeks and none after 3 weeks. Of the 29 isolates recovered in selective *Yersinia* agar, 22 (75.1%) were isolated after 1 week of cold enrichment, 6 (20.7%) after 2 weeks and 1 (3.4%) after 3 weeks.

UNITERMS: *Yersinia pseudotuberculosis*; *Yersinia* infections; Buffaloes; Carriers.

INTRODUCTION

Yersinia pseudotuberculosis has been reported as a cause of enteritis in deer[8], cattle[2,3,5,16,17,19,20]; sheep[14,18]; goats18 and pigs[1,7,18]. In Southern Brazil, yersiniosis caused by *Y. pseudotuberculosis* has emerged as an important disease in buffaloes grazing poor quality pastures, during winter and early spring[15]. The objects of this study were to measure carrier states for *Y. pseudotuberculosis* in the intestinal tract of buffaloes, and to evaluate the efficiency of Mac Conckey agar and selective *Yersinia* agar for the isolation of this bacterium after 1, 2 and 3 weeks of cold enrichment.

MATERIAL AND METHOD

To determine the number of clinically healthy buffaloes carrying *Y. pseudotuberculosis*, fecal samples from 119 healthy buffaloes, from 5 farms in Southern Rio Grande do Sul, were collected between September 1989 and August 1990. The samples were collected directly from the rectum and placed in plastic bags. Date of collectings, number of samples, age of the buffaloes and history of yersiniosis on each farm are presented in Table 1. In farm 5, fecal samples from healthy buffaloes were collected at the end of an outbreak of yersiniosis which involved 160, 7-10 month old lactating calves. Thirty of these (19.7%) had diarrhea and 5 (3.1%) died. The diagnosis of yersiniosis was confirmed by the isolation of *Y. pseudotuberculosis* from 4 affected, untreated calves.

Two grams of feces were suspended in 20 ml of brain heart infusion broth* (BHI) and held at 4°C for 3 weeks for cold enrichment. The 20 samples collected during 1989 were plated, after 1, 2 and 3 weeks of cold enrichment, onto Mac Conckey agar*. The 96 samples collected during 1989 were plated, after cold enrichment, on Mac Conckey agar and on Bacto *Yersinia* Selective Agar Base with Bacto *Yersinia* Antimicrobial Supplement* (BYSA). Colonies suspected of being *Yersinia sp.* were inoculated into triple sugar iron agar* (TSI) and incubated at 37°C for 48 hours. Isolates that produced acid, but not gas from glucose and did not produce H_2S on TSI were tested for catalase, oxidase and indol production, nitrate reduction, and production of acid from galactose, maltose, mannitol, sucrose and lactose. Isolates identified as *Yersinia pseudotuberculosis* were sent to the University of São Paulo for serological identification by the slide aglutination test.

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RESULTS

Yersinia spp. were not isolated from samples from farms 1, 2, 3 and 4. *Yersinia pseudotuberculosis* was isolated from 30 of the 34 samples collected from farm 5. It was recovered in 21 of

Table 1

Details of the collecting of feces samples from buffaloes and history of yersiniosis in 5 farms - Southern Rio Grande do Sul, September 1989 and August 1990.

Farm nº / Location (Municipality)		Collecting dates (month/year)	Age of the buffaloes	Number of samples collected	
1	Arroio Grande	9/89	NR*	11	Outbreaks of yersiniosis in 1987 and 1988
2	Rio Grande	10/89	NR	6	No history of yersiniosis
3	Capão do Leão	10/89	NR	10	Outbreak of yersiniosis in 1984
		4/90	1-2 years	10	
		5/90	Adults	23	
4	Uruguaiana	6/90	Adults	25	Outbreak of diarrhea diagnosed presumptively as yersiniosis in 1989
5	Uruguaiana	8/90	Adults	25	Samples collected at the end of an outbreak of yersiniosis
			7-10 months	9	

*No records

the 25 samples from adult, healthy females, and in all 9 samples from healthy calves. All isolates were positive for catalase, NO_3 reduction and urease, and negative for oxidase. Acid but not gas was produced from glucose, galactose, maltose and mannitol. No acid was produced from sucrose and lactose. All isolates were identified by the slide aglutination test as *Y. pseudotuberculosis* serotype III.

Of the 30 isolates from clinically healthy buffaloes 14 (46.7%) were recovered on BYSA and Mac Conckey, 1 (3.3%) only on Mac Conckey, and 15 (50%) only on BYSA. Of the 15 isolates recovered on Mac Conckey, 12 (80%) were isolated after one week of cold enrichment, 3 (20%) after 2 weeks and none after 3 weeks. Of the 29 isolates recovered on BYSA, 22 (75.1%) were isolated after 1 week of cold enrichment, 6 (20.7%) after 2 weeks, and 1 (3.4%) after 3 weeks.

DISCUSSION

Studies of healthy carriers for *Y. pseudotuberculosis* record a great variation in the rates of prevalence, going from 0[21], 0.16%[6], 0.3%[4], to 26.3%[11] in cattle, and from 0.13%[9], 0.8%[10] to 10.7%[12] in deer. However, it is very difficult to compare these results because of the wide variety of sampling and of culture techniques used. In New Zealand, Hodges; Carman[11] (1985) comparing their results (10.7% of positive samples) using BYSA, with the results obtained by Henderson; Hemmingsen[10] (1984) (0.8% of positive samples) using Mac Conckey agar, concluded that the most likely explanation for their differences were the cultural methods used. In our investigation, BYSA was twice as efficient for the detection of healthy carriers for *Y. pseudotuberculosis* in the feces as Mac Conckey agar.

The isolation of *Y. pseudotuberculosis* from 88% of the clinically healthy buffaloes on a farm where an outbreak of yersiniosis was occurring indicates that, during outbreaks, many subclinically infected were shedding the bacterium. The presence during outbreaks of a high number of healthy animals shedding *Y. pseudotuberculosis* has also been reported in cattle, and appears to be important in the epidemiology of the disease. The seasonal incidence of yersiniosis is probably due to the excretion of *Y. pseudotuberculosis* in large numbers by infected animals[17], and to the ability of the bacterium to survive and multiply in a cool, damp environment[5,17].

The negative results obtained on other farms, including the 3 with a previous history of yersiniosis (farms 1, 3, and 4), suggest the absence of healthy carriers, or their occurrence in low numbers, probably shedding the bacterium intermitently. These findings suggest that the carrier state, as evidenced by the presence of the organism in the feces, is transient. Similar findings have been reported in cattle in Australia, where *Y. pseudotuberculosis* was not isolated in lymphonodes, spleen, liver and gut from a calf killed 72 days after the experimental infection[17]. Also, in a group of 32 calves excreting *Y. pseudotuberculosis*, 19 of which were treated with oxytetracycline and 13 untreated, the bacterium was isolated in 17 of the 19 treated calves, and in all untreated ones 5 days after treatment; however, after 61 days it was recovered only from 3 untreated calves[17].

Some measures appear to be important for the control of the disease, including the parenteral treatment with antibiotics of clinically affected buffaloes and their isolation, to prevent the contamination of pastures with large number of *Yersinia.* Confining the herd to a dry paddock will help to prevent the survival of *Y. pseudotuberculosis* in the environment. It is also important to reduce, as much as possible, the stress caused by shortage of grass associated with the cold, wet weather during winter and early spring. Such stress has been associated with outbreaks of yersiniosis in buffaloes[15].

In this investigation only *Y. pseudotuberculosis* serotype III was recovered. In earlier outbreaks of yersiniosis in the State of Rio Grande do Sul, serotypes I[15] and III (Riet-Correa *et al.***, s.d.) were involved. In the State of Paraná serotype III is the only one recovered from buffaloes[13] and cattle[16,19,20].

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RESUMO

Amostras de matérias fecais de 119 búfalos clinicamente sadios, de 5 propriedades, foram colocadas em enriquecimento a 10% em infusão de cérebro e coração por 3 semanas e semeadas semanalmente em meio seletivo para *Yersinia spp* (*Yersinis* Selective Agar com *Yersinia* Antimicrobial Supplement) e em agar Mac Conckey. *Yersinia pseudotuberculosis* sorotipo OIII foi isolada somente em amostras de uma propriedade em que estava ocorrendo um surto de yersiniose. A bactéria foi recuperada de 21 amostras de um total de 25 amostras de fêmeas adultas sadias e de todas as 9 amostras de bezerros sadios. *Y. pseudotuberculosis* não foi isolada das demais propriedades, incluindo 2 naquelas em que haviam sido diagnosticados surtos de yersiniose 1 e 5 anos antes da coleta. Dos 30 isolamentos, 14 (46,7%) foram isolados nos 2 meios de cultura, 1 (3,3%) somente em agar Mac Conckey e 15 (50%) somente em meio seletivo, demonstrando a maior eficiência deste meio para a identificação de animais portadores. Dos 15 isolamentos obtidos em Mac Conckey, 12 (80%) foram isolados após 1 semana de crioenriquecimento, 3 (20%) após 2 semanas e nenhum após 3 semanas. Dos 29 isolamentos obtidos em meio seletivo, 22 (75,1%) foram isolados após 1 semana, 6 (20,7%) após 2 semanas e 1 (3,4%) após 3 semanas.

UNITERMOS: *Yersinia pseudotuberculosis*; Yersiniose; Búfalos; Portadores.

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