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ISOLATION OF *YERSINIA ENTEROCOLITICA* SEROTYPE 0 : 3 FROM CAPTIVE MONKEYS AND DOMESTIC PIGS IN ZAIRE

by

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SUMMARY — In Zaire, *Yersinia enterocolitica* serotype 0 : 3 was isolated from liver abscesses in two monkeys and from the intestine of one of them. The same organism was found in the tonsils of three healthy slaughtered pigs and in the stool of an additional animal. It is concluded that pigs may act as a natural reservoir of *Y. enterocolitica* infection in man.

KEYWORDS : *Yersinia enterocolitica*; Monkeys; Pigs; Zaire.

Introduction

Few human infections with *Yersinia enterocolitica* have been recorded in Africa. However the occurrence of this recently recognized pathogen as a cause of gastroenteritis and septicaemia in man, has been repeatedly reported in South Africa (Finlayson e.a., 1972; Rabson & Koornhof, 1973).

The only other human *Y. enterocolitica* infections reported from Africa are : a case observed in Tanger (Mollaret e.a., 1968) and eight patients described in Kinshasa, Zaire (Makulu e.a., 1969). Makulu and associates also found three healthy carriers of *Y. e.* serotype 0 : 3 during a survey of Kinshasa butchers. The authors suggested that their infection was probably derived from slaughtered animals.

The present study shows that *Y. e.* serotype 0 : 3 is present in healthy pigs, reared in the neighbourhood of Kinshasa, and that the same organism is also a potential pathogen for non-human primates.

Materials and Methods

Monkey nr 1 was an adult male *Cercocebus aterrimus*, born in Zaire, living in Nsele Zoo (60 km from Kinshasa) from the time of capture in December 1970 until his death in September 1976. Liver and intestine were sent for examination to the Veterinary Laboratory of Kinshasa.

Monkey nr 2 was a young male *Cercopithecus ascanius*, born in Zaire, living in the same Nsele Zoo from August 1974 until his death in October 1976.

Only his liver was submitted to the laboratory. The livers from both monkeys showed the same multiple abscesses. Diseased liver tissue was plated on Tryptose Agar (Difco), MacConkey Agar (Difco) and SS Agar (Difco).

This material and intestinal contents were also enriched in Selenite Broth (Difco) and Tetrathionate Broth (Difco) before inoculation on SS Agar.

The stools of 32 monkeys from the same Nsele Zoo were inoculated into Selenite Broth, before plating on SS Agar. All media were incubated at 37° C for 48 hours. The stools of 13 monkeys from Nsele Zoo and of 10 monkeys from Kinshasa Zoo were also inoculated into the enrichment medium of Wauters (Wauters, 1973a). After 48 hours at 29° C, they were plated onto SS Agar.

From June to December 1977, 132 samples of 124 different clinically healthy pigs, alive or immediately after slaughter, were examined following the method of Wauters (1973a) : four stool samples, thirty-one tongue-swabs, forty tonsillar swabs and fifty-seven specimens from tonsillar tissue, obtained from three different farms in the neighbourhood of Kinshasa.

From August to October 1978, stool samples of thirty-four healthy pigs were cultured on Yersinia Selektivagar nach Wauters, Merck 11443 (Wauters, 1973a) and incubated for two days at 29° C.

Biochemical identification of cultures was carried out by standard methods used for identification of Enterobacteriaceae (Edwards & Ewing, 1972). Biotyping was performed according to Wauters (1973b). Antibiotic sensitivity was tested by standard disk method (Bauer e.a., 1966).

Results

Y. enterocolitica was isolated in pure culture from the liver and the intestine of monkey nr 1 and from the liver of monkey nr 2. The stools of the other monkeys were all negative for *Y. enterocolitica.*

Y. enterocolitica was also isolated from the tonsils of three healthy pigs and from the stool of one healthy pig.

There were no biochemical and serological differences between the six isolates. They grew on MacConkey Agar, SS Agar, and after enrichment in Selenite Broth and Tetrathionate Broth. The biochemical characteristics of the six strains are summarized in table 1.

The strains belonged to biotype 4, serotype 0 : 3. They were fully susceptible to chloramphenicol, tetracycline, kanamycin, streptomycin, gentamicin, sulphonamide and trimethoprim, but resistant to penicillin G, ampicillin and cephalothin.

Discussion

Makulu and collaborators (1969) reported three healthy carriers of *Y. enterocolitica* serotype 0 : 3 among Kinshasa butchers and suggested an indirect transmission of *Y. enterocolitica* by meat.

Our isolation of four strains of *Y. enterocolitica*, belonging to the same serotype, from the tonsils and stool of healthy pigs, agrees with this possibility.

Several investigators have demonstrated that swine are a potential reservoir of human infection with *Y. enterocolitica.*

Wauters and coworkers (1971) succeeded in the isolation of *Y. enterocolitica* serotype 0 : 3 from the mesenterial lymphnodes and the stools of 1.05 per cent healthy slaughtered pigs in Belgium.

TABLE 1

Biochemical characteristics of the six strains of *Yersinia enterocolitica* isolated from animals in Zaire

Test	Result	Test	Result
Motility at 22° C	+	Ornithine decarboxylase	+
Motility at 37° C	–	Aesculin hydrolysis	–
Catalase	+	Fermentation of :	
Oxidase	–	glucose	+
Indole	–	saccharose	+
Hydrogen sulfide (Kligler)	–	mannitol	+
Urease	+	xylose	–
Citrate (Simmons)	–	rhamnose	–
Malonate	–	O. N. P. G.	+
Phenylalanine deaminase	–	Lecithinase	–
Lysine decarboxylase	–	DNase	–
		Gelatinase	–

In later investigations higher isolation rates were recorded by examining pig tongues derived from several butcher's shops and tonsillar tissue of swine obtained from different slaughter-houses. *Y. enterocolitica* serotype 0 : 3 was isolated from the surface of 53 per cent of pig tongues and from about 10 per cent of tonsillar tissue examined (Wauters, Pohl & Stevens, 1976; Wauters & Janssens, 1976).

Our failure to isolate *Y. enterocolitica* from tongue swabs may be related to the fact that our material was obtained immediately after slaughter, while the tongues examined by the previous investigators were obtained after several days of cold storage. In this interval *Y. enterocolitica* organisms, which are able to multiply at low temperatures, may have gained access from the tonsillar tissue to the lingual mucosa. Also, in other countries, pigs have been found infected with « human » serotypes (3 and 9) of *Y. enterocolitica* : the Netherlands (Ackermans & Hill, 1971; Esseveld & Goudzwaard, 1973; Narucka & Westendoorp, 1977), Denmark (Pedersen, 1976), Finland and Sweden (Ahvonen, Thal & Varsenius, 1973), Republic of South Africa (Rabson & Koornhof, 1973), Canada (Toma & Deidrick, 1975), Japan (Tsubokura, Otsuki & Itagaki, 1973; Zen-Yoji e.a., 1974), Algeria (Rampon, quoted by Mollaret e.a., 1964) and France (Dickinson & Mocquot, 1961). Negative surveys were recorded from France (Bourdin, 1971; Alonso e.a., 1976), Senegal (Chambron & Bourdin, 1971) and Australia (Blackall, 1977). In these countries human infections with *Y. enterocolitica* are either unknown or reputed to be rare.

The presence of human serotypes 0 : 3 and 0 : 9 in faeces and tissues of swine does not automatically incriminate those animals as a natural reservoir of human infection. In two studies however, a direct relationship between human disease and the handling of infected pigs was evident from the epidemiological context (Ahvonen e.a., 1973; Rabson & Koornhof, 1973).

Isolation of *Y. enterocolitica* from monkeys has been reported in different countries (Kageruka, Mollaret & Vercruysse, 1972; Mair, Shubert & Harbourne, 1970; McClure, Weaver & Kaufmann, 1971; Mollaret e.a., 1970; Poelma e.a., 1977). It is impossible to conclude from those and from our own observations that the lower primates constitute a natural reservoir of *Y. enterocolitica.* All these animals had been kept in captivity for long periods and in most cases the infection was of the invasive, septicaemic type, indicating that the animals were unnatural hosts. Probably their infection was derived from the contact with human faecal material or from the ingestion of infected meat.

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Isolation de *Yersinia enterocolitica* **sérotype 0 : 3 à partir de singes en captivité et de porcs domestiques au Zaïre.**

Résumé — Au Zaïre, *Yersinia enterocolitica* sérotype 0 : 3 a été isolée à partir d'abcès du foie de deux singes et à partir de l'intestin d'un d'eux. La même bactérie a été trouvée dans les amygdales de trois porcs sains abattus et dans les selles d'un autre animal.
La viande de porc constitue donc une source possible d'infection humaine par *Y. enterocolitica*.

Isolatie van *Yersinia enterocolitica* **serotype 0 : 3 uit apen in gevangenschap en uit domestische varkens in Zaïre.**

Samenvatting — In Zaïre werd *Y. enterocolitica* serotype 0 : 3 afgezonderd uit leverabcessen van twee apen en uit de darm van één onder hen. Dezelfde bacterie werd gevonden in de amandels van drie gezonde geslachte varkens en in de faeces van een ander varken.
Contact met varkensvlees is dus een mogelijke infectiebron voor menselijke infectie door *Y. enterocolitica*.

Addendum — Since this manuscript was submitted for publication two more strains of *Y. enterocolitica* biotype 4, serotype 3, have been isolated in Kinshasa. The first was found in the stool of one pig among five that were examined in a different farm. The second was isolated in pure culture at autopsy from the liver of an eight month old monkey kept as pet in a white family.

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