

Isolation of *Edwardsiella tarda* and *Plesiomonas shigelloides* from mammals and birds in Zaïre

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RÉSUMÉ

VAN DAMME (L. R.), VANDEPITTE (J.). — Isolement d'*Edwardsiella tarda* et de *Plesiomonas shigelloides* à partir de mammifères et d'oiseaux au Zaïre. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (2) : 145-151.

Au Zaïre, *Edwardsiella tarda* a été isolée à partir de dix mammifères (3 singes, 2 bovins, 1 lamantin, 2 léopards, 1 hyène, de viande de bœuf) et treize oiseaux. *Plesiomonas shigelloides*, par contre, a été trouvé chez deux bovins, un porc, un chien et neuf oiseaux. Tous les oiseaux appartenaient à des jardins zoologiques.

Les aspects épidémiologiques et bactériologiques de ces isolements sont discutés.

Mots clés : *Edwardsiella tarda* - *Plesiomonas shigelloides* - Mammifères - Oiseaux - Zaïre.

SUMMARY

VAN DAMME (L. R.), VANDEPITTE (J.). — Isolation of *Edwardsiella tarda* and *Plesiomonas shigelloides* from mammals and birds in Zaïre. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (2) : 145-151.

In Zaïre *Edwardsiella tarda* was isolated from 10 mammals (3 monkeys, 2 cattle, 1 African manatee, 1 leopard, 1 hyaena and some beef meat) and 13 birds. *Plesiomonas shigelloides*, on the other hand, was found in 2 cattle, 1 pig, 1 dog and 9 birds. All the birds were from zoological gardens.

The epidemiological and bacteriological aspects of these findings are discussed.

Key words : *Edwardsiella tarda* - *Plesiomonas shigelloides* - Mammals - Birds - Zaïre.

INTRODUCTION

The genus *Edwardsiella* is part of the family *Enterobacteriaceae* and contains one major species *E. tarda* (9). This name was proposed by EWING (1965) (16) for a collection of strains recognised as a new entity as early as 1959 and provisionally labelled « biotype 1483-59 ». Organisms with the same properties had been isolated previously by SAKAZAKI and MURATA (1962) (31) and by KING and ADLER (1964) (23).

In Zaïre, *E. tarda* (*Et*) was detected in the stool of 51 humans with infectious gastro-

enteritis, in 4 patients with wound infection and in one premature infant with rhinitis. The organism was also cultured from one out of 300 domestic lizards (8) and from 57 p. 100 of healthy zairese freshwater fish. Fish was incriminated as a possible source of human intestinal infection (36, 39).

The genus *Plesiomonas* belongs to the family *Vibrionaceae* and contains only one species *P. shigelloides* (9). This bacterium was originally described by FERGUSON and HENDERSON (1947) (17) as an atypical « paracolone », and has since been known under several other names (*Pseudomonas shigelloides*, *Aeromonas shigelloides* and *Fergusonia*

TABLE 1 : Zairese animals from which *E. tarda* and/or *P. shigelloides* were isolated

Sort of animal	Month of isolation	Origin*	Bacteriology		sites	Lesions
			Intestine	Other		
1. Cattle (meat)	IV 76	shop	?	<i>E t</i>	meat	?
2. Monkey (<i>Cercocebus albigena</i>)	IV 76	zoo a	—	<i>E t</i>	spleen liver	?
3. Crowned hawk eagle (<i>Stephanoaëtus coronatus</i>)	IV 76	zoo c	<i>E t</i>	<i>E t</i>	liver	E T
4. Crowned hawk eagle	VII 76	zoo b	<i>E t</i>	—		E
5. East African crowned Crane (<i>Balearica sp.</i>)	VI 77	zoo b	—	<i>E t</i>	liver	T
6. Monkey (<i>Cercocebus albigena</i>)	VII 77	zoo a	—	<i>E t</i>	spleen	—
7. Cow (N'Dama)	IX 77	farm 1	—	<i>E t</i>	bone marrow	?
8. Duck	IX 77	zoo a	—	<i>E t</i>	ovaries	E T
9. Crowned hawk eagle	IX 77	zoo a	—	<i>E t</i>	liver	E T
10. Seal	X 77	zoo a	<i>E t</i>	—		E T
11. Leopard	IV 78	zoo b	?	<i>E t</i>	skin scabs	?
12. Hyena	VI 78	zoo a	<i>E t</i>	—		E
13. Marabou (<i>Leptotilus crumeniferus</i>)	VIII 78	zoo a	<i>E t</i>	<i>E t</i>	lungs	E T
14. Monkey	XI 78	private	<i>E t</i>	<i>Yersinia enteroc.</i> 0:3	liver	?
15. Marabou	XI 78	zoo a	<i>P s</i>	<i>E t</i> <i>P s</i>	kidneys	E T
16. Duck	I 79	zoo b	<i>E t</i> (duoden, caecum, cloaca) <i>Salmonella chandans</i> (cloaca)	<i>P s</i>	liver	E
17. Duck (<i>Cairina moschata</i>)	X 79	zoo a	<i>P s</i>	<i>E t</i> <i>P s</i>	liver	E T
18. Cow (N'Dama)	X 79	slaughter-house	<i>E t</i>	<i>E t</i>	bile	?
19. Duck (<i>Cairina moschata</i>)	XI 79	zoo a	<i>E t</i> <i>Salmonella kisangani</i>	—		E T
20. Marabou	I 80	zoo a	<i>E t</i>	<i>Salmonella typhimurium</i> var. <i>Copenhagen</i>	liver	E
21. Duck (<i>Cairina moschata</i>)	I 80	zoo b	—	<i>E t</i>	liver heart	T
22. Leopard	IV 80	zoo b	<i>E t</i>	?		?
23. Ibis sp.	VII 80	zoo b	<i>E t</i>	<i>E t</i>	liver ?	
24. Duck	VIII 77	zoo a	<i>P s</i>	<i>P s</i>	liver	E
25. Duck (<i>Aix galericulata</i>)	VII 78	zoo b	—	<i>P s</i>	lung	E T
26. Cow (N'Dama)	VIII 78	farm 2	<i>P s</i>	?		—
27. Duck (<i>Cairina moschata</i>)	X 78	zoo a	—	<i>P s</i>	liver	E
28. Duck (<i>Anas capensis</i>)	IX 78	zoo a	<i>P s</i> <i>Salmonella</i> (28:r:—)	<i>P s</i>	liver	?
29. Duck (<i>Cairina moschata</i>)	X 79	zoo a	<i>P s</i>	<i>P s</i> pure culture	liver	E T
30. Cow (N'Dama)	XI 79	farm +	<i>P s</i>	<i>P s</i>	liver	T
31. Pig	VIII 80	slaughter-house c	?	<i>P s</i>	lung	—
32. Kite (<i>Milvus sp.</i>)	VIII 80	zoo a	<i>P s</i>	liver	T	
33. Dog	VIII 80	private	<i>P s</i>	—		E T

* Origin : Kinshasa, except zoo c, farm 1 and 2, and slaughterhouse c (all at about 100 km from Kinshasa).
Symbols : — negative ; ? unknown ; E : enteritis ; T : tissue invasion.

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shigelloides) which have now been rejected as synonyms.

In Zaïre, *P. shigelloides* (*Ps*) was isolated from the spleen of a chimpanzee, from the kidney of a dog, from the stool of 49 patients, and from the appendix and from the gall-bladder of each one patient (37, 38). VAN DAMME and VANDEPITTE (36) found *Ps* in 59 p. 100 of healthy zairese freshwater fish. Fish was considered to be a possible reservoir for human infectious diarrhoea.

We report the isolation in Zaïre of *Et* and *Ps* from a wide range of mammals and birds. Most of the strains were isolated from specimens taken during routine autopsies.

MATERIALS AND METHODS

From July 1975 to August 1980, 32 meat samples and the following animals were submitted for bacteriological examination to the government veterinary laboratory of Kinshasa : 1 223 bovines, 59 sheep, 15 goats, 106 horses, 581 pigs, 589 poultry, 125 dogs, 19 cats, 132 primates, and 155 animals from zoological gardens (2 seals, 9 leopards, 4 hyenas, 8 crowned eagles, 7 crowned cranes, 35 ducks, and 4 marabouts). Table 1 summarizes all relevant information on the animals found positive for *Et* or/and *Ps*.

Apart from nutrient agar with and without 5 p. 100 fresh defibrinated sheep blood, following selective media were used for isolation : MacConkey agar (MC Difco), SS agar (Difco), Selenite broth (SB Difco) and Tetrathionate Broth Base (TB Difco), with added iodine solution as specified by the manufacturer. Media were incubated at 37 °C. During the last years of the study SB and TB were incubated at 43 °C, and specimens were also inoculated into TB without iodine, incubated at 37 °C.

Biochemical identification of *Et* and *Ps* was carried out with conventional methods (13) and with the API 20E system.

Antibiotic sensitivity of 21 strains of *Et* and of 9 strains of *Ps* was tested by the standard disk diffusion method (6).

Optimal conditions for recovery of *Et* were investigated by inoculating one pure culture (nr.12) into different enrichment media incubated at different temperatures.

RESULTS

Et was isolated from animals nr.1 to nr.23 ; *Ps* from animals nr.15 to nr.17 and nr.24 to nr.33 (table 1). Animals nr.15 to nr.17 were positive for both micro-organisms.

Et was isolated from eight specimens only after enrichment in TB and not by direct plating or SB enrichment. From one specimen *Et* was only recovered on direct plating and not after TB enrichment. We failed to isolate *Et* from specimens taken on the following animal species : pigs, dogs, cats, horses, sheep, goats and poultry.

Ps was isolated from six specimens on direct plating only. It was never isolated after enrichment, when the direct culture was not also positive.

All our isolates of *Et* and *Ps* showed the typical biochemical properties except for one mannitol fermenting strain of *Et* (nr.13) and two ONPG negative strains of *Ps* (nrs.26 and 31). Two strains of *Et* (nrs.17 and 19) did not produce demonstrable indol on SIM medium, tryptone water and the API 20E strip, but gave a distinct positive reaction after 3 days on MIU medium (19).

All the tested *Et* strains were susceptible to chloramphenicol, trimethoprim, streptomycin, neomycin, kanamycin, tetracycline, ampicillin, and nitrofurantoin, but resistant to colistin.

The *Ps* strains were susceptible to the above antibiotics with the exception of 3 ampicillin resistant and one streptomycin resistant strain.

Profuse growth of *Et* strain nr.12 was obtained in TB with and without iodine at all temperatures (27°, 37°, 43°), while there was no growth in SB at 43 °C and only scanty growth in SB at 37 °C.

DISCUSSION

Only sporadic records of the isolation of *Et* from healthy or diseased mammals and birds have been found in the literature, and they have been summarized in table 2.

We have isolated *Et* from the bone marrow of one N'dama cow (nr.7) and from the gall-bladder and the intestine of another one (nr.18). The latter had been transported by boat on the Zaïre river to Kinshasa. It may be

TABLE 2 : Review of *E. tarda* infections in mammals and birds

Animal species (number infected)	Country or state	Reference
Cattle (1)	: ?	: (16)
Cattle (2)	: Tchad	: (14)
Pig (3)	: Philippines	: (3, 4)
Pig (1)	: Tchad	: (14)
Pig (1)	: U.S.A.	: (28)
Dog (13 of 178 exam.)	: Vietnam	: (27)
Pigeon (1)	: Tchad	: (10)
Ostrich (1)	: U.S.A.	: (41)
Australian skink (1)	: U.S.A.	: (41)
Gull (3 of 521 exam.)	: U.S.A.	: (8)
Vulture (1 of 215 birds)	: Panama	: (24)
Flamingo (10 of 60 exam.)	: France	: (7)
Sea lion (1)	: Florida zoo	: (40)
Sea lion (2)	: Oregon	: (11)
Seal (2)	: Japan	: (30)
Seal (1)	: California	: (22)
Harbor porpoise (1)	: Oregon	: (11)
Panther (1)	: Tchad	: (14)
Monkey (1 of 671 exam.)	: Panama	: (24)

hypothesized that the intestinal colonization was due to the drinking of river water during the 4 day's journey. Fish caught from the Zaïre river has been found to contain *Et* in 10 of 23 specimens examined (11).

We have isolated only one strain of *Et* from beef meat collected at a retail shop in Kinshasa (nr.1). MAKULU (8) reported that *Et* was absent from 571 beef meat samples examined in Kinshasa, while IVESON and MacKAY-SCOLLAY (21) isolated one strain from 940 samples in Australia.

Et was found in the spleen and the liver of one monkey and in the spleen of another monkey, both from the same Kinshasa zoo. The caecum of a monkey, kept as a pet in a

European family, was also positive for *Et*. In Panama, KOURANY (7) could only isolate one strain of *Et* from 671 healthy monkeys examined.

We have found *Et* in the skin scabs of a leopard with dermatitis (nr.11) but as *Escherichia coli* was also present, a faecal contamination cannot be excluded. The faeces of another leopard with diarrhoea were also positive for *Et*. The isolation of *Et* from a panther was reported in Tchad by D'EMPAIRE (4).

Our isolation of *Et* from a hyena seems to be the first on record.

Table 3 lists the different species of mammals which have been found infected by

TABLE 3 : Review of *P. shigelloides* infections in mammals

Animal species (number infected)	Country or State	Reference
Sheep (1)	: Sri Lanka	: (33)
Goat (1)	: Sri Lanka	: (33)
Cow (1)	: Sri Lanka	: (33)
Polecat (1)	: Sri Lanka	: (33)
Dog (1)	: Germany	: (5)
Dog (2)	: Japan	: (32)
Pig (?)	: Japan	: (35)
Pig (5)	: Czechoslovakia	: (1)
Monkey (1)	: Great Britain	: (12)
Puppy (1)	: Great Britain	: (12)
Cat (1)	: Great Britain	: (12)
Dog (37 of 967 exam.)	: Japan	: (2)
Cat (40 of 389 exam.)	: Japan	: (2)
Monkey (1)	: Zaïre	: (37)
Dog (1)	: Zaïre	: (37)

Ps by other investigators. We found one mention of its isolation from birds (34).

The two *Ps* positive cattle belonged to the same herd and drank water from the river Bombo, shown to contain fish frequently infected with *Ps* (11).

Ducks nrs.17 and 29 were 4 days old and belonged to a flock of 7 which all died. They lived near a pond with tadpoles.

The superiority of TB enrichment for the isolation of *Et* and of direct plating for the isolation of *Ps* is in keeping with our previous experience with fish (11). This study showed that the isolation rate of *Et* rose from 28 p. 100 after direct plating onto MC to 52 p. 100 after enrichment in TB without iodine at 37 °C. In contrast the isolation rate of *Ps* was 78 p. 100 on direct plating, and fell to 27 after enrichment in the same broth.

The failure of *Et* to multiply in SB, already shown by IVESON (6), was confirmed by our *in vitro* studies with strain nr.12. The superiority of the TB over SB for the recovery of *Et* had also been evident from our work on fish.

The only mannitol fermenting strain of *Et* was isolated from a marabout. Typical mannitol negative cultures were isolated from the lungs and the small intestine of the same animal (nr.13). Mannitol fermenting variants of *Et* have already been described by MUYEMBE (26), VAN DAMME (11), and BAYLET (2). Strains, isolated by the latter investigators differed also in other respects and were considered as candidates for a new species (5).

All tested *Et* strains were resistant *in vitro* against colistin, a diagnostic test previously described by MAKULU (8) and MUYEMBE (26). In contrast, 20 p. 100 of the fish isolated of *Et* were susceptible to colistin (11).

From an extensive review of the literature, from our previous findings in freshwater fish and human in Zaïre and Mali, and from our present observations a tentative picture emerges concerning the ecological and epidemiological significance of *Et* and *Ps*. Both organisms seem to belong to the commensal intestinal flora of freshwater fish and perhaps of some other cold blooded waterdwelling animals as reptiles and amphibians. This explains their occasional isolation from natural waters. Their sporadic presence in animal faeces and even in the bile probably reflects transient and generally innocuous

colonization of the digestive tract with microorganisms derived from fish, water or water-contaminated food. A majority of the animal species which have been found infected with *Et* or *Ps* live in or close to water and are regular or casual fish-eaters. This is particularly true for birds as ducks, ibis, marabouts and cranes, which feed predominantly on river- and lake-fish. Birds were the only animals in our study found simultaneously infected with *Et* and *Ps* (nrs.15, 16 and 17). At least in some animals the history suggests a causal relationship with severe enteritis as in the ostrich, infected with *Et*, described by WHITE (41) and in some of our cases infected with *Et* or *Ps*.

At least in some of the reported observations the invasive nature of *Et* and *Ps* cannot be denied, although there is no indication that either species acts as a primary pathogen in healthy hosts. There is a scarcity of data on this point and the experimental inoculation of *Et* in mice and pigeons by CHAMOISEAU (3) has produced conflicting results.

The situation in mammals and birds therefore seems to be very similar to what has been observed in man, where both bacterial species may determine either asymptomatic colonization of the gut or enteritis, according to the infecting dose. Deep infections have been relatively rare with *Et* and frankly exceptional with *Ps*. As already demonstrated in an recent review (10), the great majority of systemic infections with *Et* in man occurred in patients suffering from debilitating illness.

More careful clinical observations and prospective epidemiological investigations are needed to clarify the real significance of *Ps* and *Et* for mammals and birds.

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RESUMEN

VAN DAMME (L. R.), VANDEPITTE (J.). — Aislamiento de *Edwardsiella tarda* y *Plesiomonas shigelloides* en mamíferos y aves en Zaire. *Rev. Elev. Méd. vét. Pays trop.*, 1984, **37** (2) : 145-151.

En Zaire, se aisló *Edwardsiella tarda* a partir de diez mamíferos (3 monos, 2 bovinos, 1 manatí, 2 leopardos,

1 hiena, de carne de vaca) y de 13 aves. En cambio, se encontró *Plesiomonas shigelloides* en 2 bovinos, 1 cerdo, 1 perro y 9 aves. Todos los aves pertenecían a parques zoológicos. Se discuten los aspectos epidemiológicos y bacteriológicos de estos aislamientos.

Palabras claves : *Edwardsiella tarda* - *Plesiomonas shigelloides* - Mamíferos - Aves - Zaire.

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