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CHARACTERISTICS OF *YERSINIA* SPP. ISOLATED FROM WILD AND ZOO ANIMALS

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ABSTRACT: Thirteen strains of *Yersinia* spp. were isolated at the Rome zoo and at Castelporziano, a game preserve near Rome. The strains were tested for calcium dependency, autoagglutination, heat-stable toxin production, 50% minimum lethal dose in mice (LD_{50}), pyrazinamidase activity and content of plasmids by electrophoresis in agarose gel. The former three tests were negative for all strains, the LD_{50} was always $\geq 1 \times 10^{7.6}$ CFU/ml and pyrazinamidase activity was positive for all strains. Electrophoresis revealed the presence of two plasmids of 27 and 66 megadaltons (MDa) in the two strains of *Y. enterocolitica* of serotype 027 isolated from animals in the zoo. The two strains of the same species and serotype, isolated from wild animals harboured a 42-MDa plasmid. A small plasmid of 2 MDa was found in two strains of *Y. enterocolitica* of serotype 07,8 from two subsequent samples of a zoo animal.

Key words: *Yersinia* spp. strains, zoo animals, wild animals, pyrazinamidase activity, electrophoresis, toxin production, isolation.

INTRODUCTION

Yersinia enterocolitica and *Y. enterocolitica*-like organisms are widely distributed in nature. They can be isolated from humans, animals, food and environmental samples in every country in which they have been sought (World Health Organization, 1983a).

Yersinia spp. infections are reported with increasing frequency. The epidemiology of the infection, the distribution of the serotypes, and the importance of transmission between animal and man need to be more fully understood. Previous isolations of *Yersinia* spp. strains in Italy from wild animals (Pagano et al., 1985) prompted us to conduct the present study to determine the prevalence of *Yersinia* spp. in wild and zoo animals and to compare the characteristics of *Yersinia* spp. strains isolated from animals in two different habitats in Italy.

MATERIALS AND METHODS

Study area and sampling

Two investigations were conducted. The first was at the Rome zoo in Spring 1983. The zoo consists of 17 ha of land and contains 1,300 animals. Animals examined included two eastern gray kangaroos (*Macropus giganteus*), five brown capuchins (*Cebus apella*), six green monkeys (*Cercopithecus aethiops*), three Japanese macaques (*Macaca fuscata*), one black macaque (*Macaca maura*), three rhesus macaques (*Macaca mulatta*), one gorilla (*Gorilla gorilla*), one chimpanzee (*Pan troglodytes*), seven gray wolves (*Canis lupus*), six red foxes (*Vulpes vulpes*), two polar bears (*Ursus maritimus*), seven lions (*Panthera leo*), three jaguars (*Panthera onca*), four leopards (*Panthera pardus*), three black panthers (*Panthera pardus*), two tigers (*Panthera tigris*), two giraffes (*Giraffa camelopardis*), six yaks (*Bos grunniens*), one chamois (*Rupicapra rupicapra*), four elands (*Tragelaphus oryx*), and two greater kudus (*Tragelaphus strepsiceros*). One to seven animals were housed in each cage.

In Autumn 1983 and Spring 1984, animals were studied at Castelporziano Game Preserve. The area extends over 5,012 ha of land and has about 300 red foxes (*Vulpes vulpes*), approximately 1,000 wild boar (*Sus scrofa*), 200 fallow deer (*Cervus dama*), 300 roe deer (*Cervus capreolus*) and many other species including porcupines (*Hystrix cristata*), weasels (*Mustela nivalis*), pine martens (*Martes martes*), badgers (*Meles meles*), and Hermann's tortoises (*Testudo hermanni*).

Twenty-two fecal samples from apparently healthy animals belonging to 21 species, were collected at the Rome zoo. About 2 mo later second samples were obtained from cages of animals which proved to be positive for *Yersinia* spp.

Thirty-three solid fecal samples from wild boar, red fox, fallow deer, mustelids and roe deer were collected at Castelporziano. Initially, 26 samples were collected in Autumn; at the

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second sampling, in Spring, seven were collected.

Fecal samples were collected by sterile spatules from the cage floor at the zoo and from the ground in the game preserve. Only fresh samples not contaminated with soil were collected. Fecal samples were transported to the laboratory in sterile plastic bags or in containers with Cary Blair transport medium (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, Maryland 21030, USA) (Cary and Blair, 1964).

Isolation and characterization

Specific enrichment for *Yersinia* spp. was performed in buffered peptone (Difco Laboratories, Detroit, Michigan 48232, USA) water at pH 7.4 for 21 days at 4 C followed by isolation onto MacConkey agar (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, Maryland 21030, USA) supplemented with 0.3% tween 80 (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, Maryland 21030, USA).

Colony characteristics of *Yersinia* spp. were identified by API 20 E (API System S.A., La Balme Les Grottes, 38390 Montalieu Vercieu, France). For a better characterization of species API 50 CH (API System S.A., La Balme Les Grottes, 38390 Montalieu Vercieu, France) was utilized. The biotype was determined according to the scheme of Bercovier et al. (1980). The serotype was determined using 0 antisera prepared in our laboratory by method of Wauters et al. (1971, 1982). Susceptibility to antimicrobial agents was determined by the disc-agar diffusion method (Bauer et al., 1966) using the following antibacterial drugs: ampicillin (AM, 10 μg), cephalothin (CF, 30 μg), chloramphenicol (C, 30 μg), colistin (CL, 10 μg), gentamicin (GM, 10 μg), kanamycin (K, 30 μg), nalidixic acid (NA, 30 μg), streptomycin (S, 10 μg), sulfisoxazole (G, 2.0 mg), tetracycline (TE, 30 μg), tobramycin (TM, 10 μg) and trimethoprim-sulfamethoxazole (SXT, 25 μg). The isolates were examined by the following tests: 50% minimum lethal dose (LD_{50}) in mice by the method of Smith et al. (1981). The LD_{50} was calculated from cumulative mortality data by the method of Reed and Muench (1938). Calcium dependency (CAD) was determined on calcium-deficiency differential plating medium (Higuchi et al., 1959). The autoagglutination test (AA) was performed by the method of Laird and Cavanaugh (1980). Heat stable toxin (ST) production was tested in the suckling mouse by the method of Dean et al. (1972). Pyrazinamidase activity (PYZ) was detected by the method of Kandolo and Wauters (1985). Hydrolysis of pyrazinamidase was indicated by brownish-pink colour in the presence of ferrous salts. Plasmids were determined by the method of Kado and Liu (1981). The molecular size of plasmids was estimated by comparing their electrophoretic mobilities with those of plasmids of known molecular weights expressed in megadaltons (MDa). These included: R27 (112 MDa), R16 (69 MDa), R471 (52 MDa), RP4 (36 MDa), pBR 322 (2.84 MDa) kindly provided by Dr. V. Falbo (Laboratorio di Ultrastrutture, Istituto Superiore di Sanità, Rome 00161, Italy).

RESULTS AND DISCUSSION

Strains of *Yersinia* spp. were isolated in 27% of the fecal samples from zoo animals and 15% of those from wild animals. All the strains were grown in enrichment cultures. Species and strains and their biological characteristics are listed in Table 1.

Four strains of *Y. enterocolitica* were isolated from the black panther, tiger, red fox and brown capuchin, respectively, from 22 fecal samples examined from the first collection at the Rome zoo. Second samples from these four animals were collected, of which three were positive. A single sample was negative, and one grew two different strains of *Yersinia* spp.

Five different strains of *Yersinia* spp. were cultured from five of 33 samples from wild animals. The five positive samples were collected in Autumn.

All the strains from zoo animals belonged to biotype 1. Seven strains were *Y. enterocolitica* including two serotype 027, four 07,8, and one $010K_1$-34; one strain was *Y. intermedia* serotype 014-16,29.

Four strains from wild animals were identified as *Y. enterocolitica* and one as *Y. kristensenii*. Two strains were biotype 1 and serotype 027, one was biotype 2 and serotype $010K_1$-34 and one was biotype 3, non-agglutinable (NAG). Also, the *Y. kristensenii* isolate was found to be NAG.

None of the strains isolated at zoo or game preserve were serotypes considered to be pathogenic for man. The two NAG strains were sensitive to all antimicrobics tested; all the other strains showed only usual resistance to AM and CF. The suckling mouse test indicated production of ST in all the tested strains of *Y. enterocolitica*.

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TABLE 1. Characteristics of *Yersinia* spp. isolates from animals in Rome, Italy.

Place	Host	*Yersinia*		PYZ[a]	Esc[b]	Antimicrobic resistance	ST[c]	LD_{50}[d]	Plasmid size (MDa)
		Species	Bioserotype						
Zoo	black panther	Y.e.[e]	1/027	+	+	AM, CF[f]	+	10^{9}	27, 66
	tiger	Y.e.	1/07,8	+	+	AM, CF	+	$10^{8.7}$	ND[k]
	red fox	Y.e.	1/027	+	+	AM, CF	+	10^{9}	27, 66
	brown capuchin monkey	Y.e.	1/07,8	+	+	AM, CF	+	$10^{7.6}$	2
	brown capuchin monkey	Y.e.	1/07,8	+	+	AM, CF	+	$10^{8.3}$	2
	black panther	Y.e.	$1/010K_1$-34	+	+	AM, CF	+	$10^{8.3}$	ND
	red fox	Y.e.	1/07,8	+	+	AM, CF	+	$10^{8.2}$	ND
	red fox	Y.i.[i]	1/014-16,29	+	+	AM, CF	−	$10^{8.5}$	ND
Game preserve	fallow deer	Y.e.	$2/010K_1$-34	+	+	AM, CF	NT[j]	NT	ND
	mustelid	Y.e.	3/NAG[h]	+	−	−	NT	NT	ND
	red fox	Y.k.[g]	NAG	+	−	−	NT	NT	ND
	wild boar	Y.e.	1/027	+	+	AM, CF	NT	$10^{8.7}$	42
	mustelid	Y.e.	1/027	+	+	AM, CF	NT	$10^{8.8}$	42

[a] Pyrazinamidase activity; +, positive; −, negative.
[b] Esculin hydrolysis; +, positive; −, negative.
[c] Heat stable toxin production; +, produced; −, not produced.
[d] LD_{50}, 50% lethal dose in mice.
[e] Y.e., *Yersinia enterocolitica*.
[i] Y.i., *Yersinia intermedia*.
[g] Y.k., *Yersinia kristensenii*.
[h] NAG, not agglutinable.
[f] AM, ampicillin; CF, cephalothin; −, not resistant.
[j] NT, not tested.
[k] ND, none detected.

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Yersinia intermedia isolate did not produce ST.

The LD_{50} of all isolates was $\geq 1 \times 10^{7.6}$ CFU/ml. Furthermore, all the strains showed pyrazinamidase activity, associated with the positive reaction of esculin hydrolysis in all but the two NAG isolates from wild animals.

Four types of plasmids with different molecular weights were detected. Those from two strains of serotype 027 isolated from the red fox and from the panther at the zoo, were similar; both harboured two plasmids of 27 and 66 MDa each. The two 027 strains isolated from wild animals, similar *inter eos*, differed from the 027 isolated from the zoo which had a single plasmid of 42 MDa. One of these strains was isolated from the wild boar and the other from the weasel. Only one small plasmid of 2 MDa was detected in the two isolates from the brown capuchin from the four strains of serotype 07,8 (Table 1).

Calcium dependency and autoagglutination were negative for all strains.

Our findings agree with those of previous authors who isolated *Yersinia* spp. strains from both wild and zoo animals (Obwolo, 1976; Poelma et al., 1977; Pagano et al., 1985). The possible role of animals as reservoirs of human infections, as well as the mode of transmission of *Yersinia* spp. among animals and to humans, remains to be elucidated. The epidemiology of *Yersinia* spp. is unknown also. Because the same serotypes isolated in human infections were isolated also from healthy free-ranging and captive wild as well as domestic animals (Mair, 1973; Hacking and Sileo, 1974; Fantasia-Mazzotti and Giraldi, 1983; Fantasia et al., 1985), they can play an important role in the epidemiology of yersiniosis. Thus, public health measures like those for the prevention and control of zoonotic salmonellosis should be adopted (World Health Organization, 1983b).

From plasmid analysis, we noted that the zoo strains of serotype 07,8 belonged to two different clones. The strains of serotype 027 circulating in zoo animals belonged to the same clone; the strains of serotype 027 circulating in the Castelporziano Game Preserve belonged to another clone. All the isolates were negative for pathogenic characteristics; the lack of virulence of the isolates is indicated also by the lack of a characteristic "virulence" plasmid such as 42–48 MDa in all but two strains (Kay et al., 1982; Skurnik et al., 1983; Kapperud et al., 1985). The occurrence of 42 MDa plasmids in the two strains of serotype 027 isolated from wild animals without other positive tests for virulence poses a problem that should be examined to establish whether or not such plasmids are similar to those associated with virulence.

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