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Panoramic view of the occurrence of *Yersinia* species other than *Y. pestis* in Brazil

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ABSTRACT

Data on the occurrence of *Yersinia* species. other than *Y. pestis* in Brazil are presented. Over the past 40 years, 767 *Yersinia* strains have been identified and typed by the National Reference Center on *Yersinia* spp. other than *Y. pestis*, using the classical biochemical tests for species characterization. The strains were further classified into biotypes, serotypes and phagetypes when pertinent. These tests led to the identification of *Yersinia* cultures belonging to the species *Y. enterocolitica, Y. pseudotuberculosis, Y. intermedia, Y. frederiksenii* and *Y. kristensenii.* Six isolates could not be classified in any of the known *Yersinia* species and for this reason were defined as Non-typable (NT). The bio-sero-phagetypes of these strains were diverse. The following species of *Yersinia* were not identified among the Brazilian strains by the classical phenotypic or biochemical tests: *Y. aldovae, Y. rhodei, Y. mollaretti, Y. bercovieri and Y. ruckeri.* The *Yersinia* strains were isolated from clinical material taken from sick and/or healthy humans and animals, from various types of food and from the environment, by investigators of various Institutions localized in different cities and regions of Brazil.

Keywords: Yersinia spp.; occurrence; Brazil

INTRODUCTION

The bacterial genus *Yersinia* belongs to the family *Enterobacteriaceae* and comprises 12 species. The species *Y. pestis, Y. pseudotuberculosis* and *Y. enterocolitica* are associated with human and animal diseases. *Y. pestis* causes plague, *Y. pseudotuberculosis* causes mesenteric lymphadenitis and septicemia and *Y. enterocolitica,* which is the most prevalent among humans, causes mainly a gastrointestinal syndrome varying from acute enteritis to mesenteric lymphadenitis. *Y. intermedia, Y. frederiksenii, Y. kristensenii, Y. aldovae, Y. rhodei, Y. molaretii* and *Y. bercovieri* are considered to be environmental species. *Y. ruckeri* is associated to a fish disease (Sulakvelidze, 2000; Bottone et al., 2005). The newest member of the genus is *Y. aleksiciae* previously included in the species *Y. kristensenii* (Sprague & Neubauer, 2005).

Usually, *Yersinia* species are identified and typed by their biochemical and serological characteristics and by determining of their phagetypes. The strains of some species like *Y. enterocolitica*, *Y. pseudotuberculosis* and *Y. intermedia* can be differentiated into different biotypes: there are five biotypes of *Y.enterocolitica,* biotype 1 being subdivided into 1A and 1B, four of *Y. pseudotuberculosis* and eight of *Y. intermedia*. Specifically, the *Y. enterocolitica* biotypes are of variable epidemiological and clinical importance (Bottone, 1999; Robins-Browne, 2001). Recently, molecular techniques have also been used to characterize these species (Tenover et al., 1997; Olive & Bean, 1999; Salyers & Whitt, 2002).

In order to cause disease, pathogenic *Yersinia* needs a group of virulence factors of chromosomal and plasmid origin that enable the microorganism to clonize the host and escape its specific and nonspecific immune response (Robins-Browne, 2001).

Molecular genetics studies on *Y. enterocolitica* and *Y. pseudotuberculosis* have emphasized the importance of a virulence plasmid (pYV) which encodes various virulence genes, as well as specific chromosomal genes that mediate cell invasion (genes *inv* and *ail*), iron capture (genes of the high-pathogenicity island), enterotoxin Yst production (gene *yst*), among other effects (Carniel, 1995). The remaining eight species, other than *Y. pestis,* have not been extensively studied and, because of the absence of classical *Yersinia* virulence markers, are generally considered to be environmental and non-pathogenic. However, an increasing number of these non-pathogenic species have been isolated from sick humans, raising the question of their possible pathogenicity (Sulakvelidze, 2000).

In 1980 a National Reference Center on *Yersinia* spp.(except for *Y. pestis*), was established in Brazil in the Microbiology Laboratory at UNESP, in Araraquara, state of São Paulo, in collaboration with the *Yersinia* International Center of the World Health Organization at the Pasteur Institute in Paris, France.

The present review was prepared in order to provide a summary of the data on the occurrence of *Yersinia* species other than *Y. pestis* in Brazil, assessed during the research conducted by our group at the *Yersinia* spp. Reference Laboratory.

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YERSINIA IN BRAZIL

In Brazil, *Yersinia* infections and occurrence are not reported as frequently as in other countries.

The first isolations of *Y. enterocolitica* in Brazil were from hepatic abscesses of captive marmosets at São Paulo Zoo and were described by Giorgi et al. (1969) and by Mollaret et al. (1970).

This microorganism was associated with human pathology for the first time in Brazil in 1976, when it was isolated from a case of pseudoappendicitis in the city of Araraquara, state of São Paulo, by Pizsolitto et al. (1979).

Since that time, strains of *Y. enterocolitica* have been isolated from human and animal clinical material, food and environment in this country (Falcão, 1981; 1987; 1991).

It should be mentioned that our laboratory has also performed two serological investigations, using human and pig sera. In the first survey, antibodies against *Y. enterocolitica* O:3, O:8 and O:9 and *Y. pseudotuberculosis* O:1 and O:3 were sought in 1069 specimens of human serum, from different patients in several regions of the state of São Paulo, and it was shown that about 1% of all sera tested were positive for anti-*Y. enterocolitica* O:3 or O:9 (Lopes & Falcão, 1980). In the other serological survey, anti-*Y. enterocolitica* O:3, O:5 and O:9 antibodies were screened in 1799 sera from pigs in herds of São Paulo, Paraná and Santa Catarina states and the results showed that about 21.9% of all the sera were positive for one of these antibodies, indicating the probable importance of pigs as a reservoir of *Yersinia* spp. in Brazil (Falcão et al.,1980).

Early reports of *Y. pseudotuberculosis* in Brazil were made by Hofer et al. (1979), who isolated the microorganism from a healthy rodent, and by Barcellos et al. (1980), who isolated the bacteria from the feces of pigs with diarrhea. It is important to stress that until now, to our knowledge, *Y. pseudotuberculosis* has been isolated only from animals and never from humans in Brazil (Martins et al., 1998; 2001).

Strains of *Y. intermedia, Y. frederikseni* and *Y. kristensenii*, classified as non-pathogenic, have been isolated in Brazil from food and the environment and some from humans and animals (Falcão, 1981; 1987; 1991; Falcão et al., 2004).

These *Yersinia* strains have been isolated from 1968 until now (40 years) and have been received by the *Yersinia* Reference Center on *Yersinia* spp. other than *Y. pestis* for confirmation and typing. All strains confirmed as *Yersinia* spp. were also biotyped, serotyped and phagetyped, except in the case of *Y. pseudotuberculosis* strains, which were only bio-serotyped. The confirmation and the bio-sero-phagetyping of these *Yersinia* strains were carried out mostly at the *Yersinia* Reference Laboratory mentioned above, except for a few strains that were bio-sero-phagetyped at the Pasteur Institute in Paris. Strains were isolated from a variety of sources, cities and regions of Brazil.

YERSINIA STRAINS OTHER THAN *Y. PESTIS* OF THE NATIONAL REFERENCE CENTER COLLECTION

A total of 767 *Yersinia* strains have been isolated from human, animal, food and environmental sources in this country and belong to the culture collection of the National Reference Center on *Yersinia* spp. other than *Y. pestis*, previously located at the Bacteriology Laboratory of the School of Pharmaceutical Sciences, UNESP University, in the city of Araraquara, state of São Paulo and now transferred to the Bacteriology Laboratory of the Department of Clinical Analysis, Bromatology and Toxicology of the School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP).

These 767 *Yersinia* strains were identified according to Aleksic & Bockemühl (1999) as *Y. enterocolitica* (357 strains), *Y. intermedia* (199 strains), *Y. frederiksenii* (87 strains), *Y. kristensenii* (13 strains) and *Y. pseudotuberculosis* (105 strains). Additionally, six strains, with biochemical characteristics different from those of the known *Yersinia* species, were typed but could not be classified in any of the currently known species and, for this reason, were named as Non-typable (NT).

None of the Brazilian strains were identified by the biochemical tests as *Y. aldovae, Y. mollaretti, Y. bercovieri, Y. rhodei* or *Y. ruckeri*. However, the Reference Laboratory identified one strain of *Y. rhodei* isolated in Argentina from recreational water (Duaigues et al., 1992).

Table 1 summarizes our data on the general characteristics of the 767 Brazilian *Yersinia* spp. strains other than *Y. pestis*, of different species and sources, isolated by investigators of various cities and regions of this country.

The general characteristics of *Yersinia* strains of different origins, received by the Reference Laboratory, belonging to the species *Y. enterocolitica, Y. pseudotuberculosis, Y. intermedia, Y. frederiksenii* and *Y. kristensenii* are presented separately in Tables 2 to 9. Additionally, the characteristics of the six non-typable (NT) *Yersinia* strains are presented in Table 10.

Table 2 shows the distribution of the bio-sero-phagetypes, sources of isolation, places of origin and year of reception of the 142 *Y. enterocolitica* strains of human origin. Equivalent data on the 75 *Y. enterocolitica* strains isolated from animals, 73 *Y. enterocolitica* strains isolated from food and 67 *Y. enterocolitica* strains isolated from the environment are given in details in Tables 3 and 4.

Table 5 shows similar data for *Y. intermedia* isolated from human (one strain) animals (two strains) and environment (64 strains). Data on *Y. intermedia* isolated from food (132 strains) is displayed in Table 6.

The characteristics of the *Y. frederiksenii* isolated from humans (two strains), animals (five strains), food (70 strains) and environment (10 strains) are presented in Table 7.

Table 8 presents the characteristics of the *Y. kristensenii* isolated from animals (one strain), food (nine strains) and environment (three strains).

Table 1 - Summary of the characteristics of the 767 *Yersinia* strains confirmed and bio-sero-phagetyped at National Reference Center on *Yersinia* spp. in Brazil, according to their origin, species and source of isolation.

Number of strains/Origin	*Yersinia* species	Number of strains/ Source of Isolation
145/ Human[1]	142 *Y. enterocolitica* 2 *Y. frederiksenii* 1 *Y. intermedia*	138 feces normal feces(15) diarrheic feces(123) 2 ascitic fluid 1 throat swab 2 blood 1 hand-wash 1 maxillar bone
188/ Animal[2]	75 *Y. enterocolitica* 5 *Y. frederiksenni* 2 *Y. intermedia* 105 *Y. pseudotuberculosis* 1 *Y. kristensenii*	161 bovine, buffalo, rat, pig, dog and capybara feces 1 buffalo organs 2 bovine intestinal loops 1 capybara vaginal secretion 3 buffalo mesenteric ganglia 9 bovine large intestine material 5 bovine small intestine material 5 marmoset hepatic abscess 1 monkey hepatic abscess
289/ Food[3]	73 *Y. enterocolitica* 132 *Y. intermedia* 70 *Y. frederiksenii* 9 *Y. kristensenii* 5 *Yersinia* Non-typable[a]	144 milk 67 meat 28 cheese 3 hamburger 11 sausage 6 vegetable 1 ice 11 oyster 6 meat balls 10 chicken pieces 2 bovine liver
145/ Environment[4]	67 *Y. enterocolitica* 64 *Y. intermedia* 3 *Y. kristensenii* 10 *Y. frederiksenii* 1 Non-typable[a]	102 water 43 sewage

[1] The 145 *Yersinia* strains of human origin were isolated by: Piszolitto et al. (1979), Stumpf et al. (1978), Fontes et al. (1978), Ferreira et al. (1979), Toledo & Falcão (1980), Decarlis et al. (1982), Martinez & Moura (1984), Martinez & de Bonis (1985), Nunes & Ricciardi. (1986), Suassuna et al. (1986), Kitagawa et al. (1989), Ceccarelli et al. (1990), Perroni et al. (1995), Bonifácio da Silva et al. (1997), Almeida et al. (1999); Medeiros et al. (2001); LR Trabulsi (1980)* in São Paulo-SP; M Magalhães & D Montenegro (1981, 1982)* in Recife-PE; MR Toledo (1981, 1983, 1989)* in São Paulo-SP; B Bertschinger (1988)* in Porto Alegre-RS; M Martinez (1984, 1988)* in São Paulo-SP; EL Pizzolitto (1986, 1991, 1992, 1993, 1995)* in Araraquara-SP; A Souza (1993)* in Presidente Prudente-SP; MI Medeiros (2003, 2004, 2006); D.Lima (2007)* and ME Bonifácio da Silva (2008)* all in Ribeirão Preto-SP

[2] The 188 *Yersinia* strains of animal origin were isolated by Giorgi et al. (1969), Mollaret et al. (1970), Simon et al. (1975), Genovez et al. (1980), Barcelos et al. (1980), Barcellos & Pestana de Castro (1981), Oliveira et al. (1983), Warth et al. (1984), Suzumura (1984), Ferreira et al. (1985), Warth et al. (1985), Nunes & Ricciardi (1986), Saridakis et al. (1988), Riet-Corrêa et al. (1990), Warth (1990), Estima et al. (1996), Nogueira (1998), in Botucatú-SP; MEA. Klüppel (1985)* in Curitiba-PR; MRST Decarlis (1970, 1975)* and FJP Listone (1997) *both in Botucatu-SP.

[3] The 289 *Yersinia* strains of food origin were isolated by Uboldi Eiro et al. (1984, 1986, 1988), Tibana et al. (1987), Landgraf & Falcão (1987), Warnken et al. (1987), Leite et al. (1988), Moro & Nunes (1992), Landgraf et al. (1993), Tassinari et al. (1994); Warnken et al. (1997, 2001), Nobrega et al. (2000), Falcão et al. (2002).

[4] The environmental *Yersinia* strains (145) were isolated by Freitas et al. (1987), by MT Martins & DMF Varanda (1982)* in São Paulo-SP

[a] The six atypical *Yersinia* strains were isolated by Landgraf & Falcão (1987), Freitas et al. (1987), Tassinari et al. (1994).

*= not published

The characteristics of the 105 *Y. pseudotuberculosis* strains isolated from animals are presented in Table 9.

Table 10 shows the characteristics of the six atypical (Non-typable) *Yersinia* strains isolated from food (five strains) and from the environment (one strain).

FURTHER COMMENTS

The great majority (97.9%) of the *Yersinia* strains isolated from humans in Brazil are *Y. enterocolitica,* the rest being only 0.7% *Y. intermedia* and 1.4% *Y. frederiksenii.* Most of the *Y. enterocolitica* strains (88.4%), belong to biotype 4 and serogroup O3: which is also the prevalent

Table 2 – Distribution of types of 142 *Y. enterocolitica* (Ye) strains isolated from humans according to their source of isolation, origin and year of reception.

No. of Strains	Bio-sero-phagetypes	No. of Strains/ Source	Origin [a]	Year of reception
122	Ye4 /O:3/VIII	2/ascitic fluid	FCF-SP; FMRP-RP	1984, 1992
		2/blood	FCF-SP; IAL-RP;	1992, 2004
		10/normal feces	UFRJ-RJ; UNESP-Ar UNIFESP-SP	1980, 1982, 1985, 1993
		107/diarrheic feces	FCF-Ar; LIAC-SP; UFP-PE, CEMI-PE; LBCL-SP UNIFESP-SP; FCF-SP; FCFRP-RP UNESP-Bot; UFRJ-RJ; FMRP-RP; FM-SJRP	From 1976 to 2008
		1/maxillar bone	FMRP-RP	2007
4	Ye4/O:3/IXa	4/diarrheic feces	FMRP-RP	1987, 1989, 1991
7	Ye1A/O:5/Xz	1/throat swab	UNIFESP-SP	1979
		5/normal feces	UFRJ-RJ	1985
		1/diarrheic feces	LIAC-SP	1982
5	Ye2/O:5/Xz	5/diarrheic feces	UFRJ- RJ	1979
1	Ye3/O:5,27/Xz	1/hand-wash	UFRJ-RJ	1984
2	Ye1A/O:6,30/Xz	2/diarrheic feces	UERJ-RJ; IAL-RP	1980, 1999
1	Ye1A/O:8,19/Xo	1/diarrheic feces	IAL-RP	2000

[a] FCF-SP:Faculdade de Ciências Farmacêuticas-USP; FCFRP:Faculdade de Ciências Farmacêuticas de Ribeirão Preto-USP; FCF-Ar.:Faculdade de Ciências Farmacêuticas-UNESP; USP:Universidade de São Paulo; IAL:Instituto Adolfo Lutz; UFRJ:Universidade Federal do Rio de Janeiro; UNESP:Universidade Estadual Paulista; LIAC:Laboratório Integrado de Análises Clínicas; UFP:Universidade Federal de Pernambuco; CEMI:Centro de Microbiologia e Imunologia; UNIFESP:Universidade Federal de São Paulo; UERJ:Universidade Estadual do Rio de Janeiro; Ar:Araraquara; Bot:Botucatu; RP:Ribeirão Preto; FMSJRP:Faculdade de Medicina de São José do Rio Preto; LBCL:Laboratório Bio-Ciências Lavoisier. FMRP:Faculdade de Medicina Ribeirão Preto-USP.

type around the world (Bottone,1999; Robins-Browne, 2001). The others bio-serotypes found in the strains of human origin were: 1A/O:5, 2/O:5, 3/O:5,27, 1A/O:6,30 and 1A/O:8,19. It is important to mention that none of the human isolates of *Y. enterocolitica* was classified as biotype 1B, which is linked to high virulence (Carniel, 1999). Nevertheless, it must be pointed out that *Y. enterocolitica* 1A/O:5, which is considered to be a non-pathogenic environmental strain (Robins-Browne, 2001) was isolated from diarrheic feces in São Paulo in 1982 (LR Trabulsi, data not published). Also, a strain isolated from diarrheic feces in Argentina was typed as *Y. enterocolitica* 1A/O:5 by the National Reference Center in *Yersinia* spp. in Brazil (Paz et al., 1998).

The majority of the 188 *Yersinia* isolated from sick and healthy animals, were classified as *Y. pseudotuberculosis* (55.85%) and *Y. enterocolitica* (39.9%). The *Y. enterocolitica* strains were mostly of bio-serotypes 4/O:3 and 1A/O:5. In the latter group, the bacteria were isolated from dog feces, in some cases from diarrheic feces (Nunes&Ricciardi,1986). The other species found were *Y. intermedia* (2.66%), *Y. frederikisenii* (2.66%) and *Y. kristensenii* (1.06%). It is important to emphasize that *Y. pseudotuberculosis* has been isolated only from animals and never from humans in Brazil, as showed by data from our group (Martins et al., 1998; 2001).

Y. enterocolitica strains isolated from food in Brazil belong to bio-serotypes that are usually not linked to human and animals infections. As reported here, besides the species *Y. enterocolitica* (25.25%), a large number of *Y. intermedia* (45.67%), *Y. frederiksenii* (24.22%), a few *Y. kristensen*i (3.12%) and Non-tipable (1.73%) were recovered from different kinds of food. This study showed a high occurrence of *Yersinia* species especially in milk, probably indicating improper production conditions and/or post-pasteurization contamination.

A total of 145 *Yersinia* strains isolated from the environment were classified as *Y. enterocolitica*

Table 3 – Distribution of types of 75 *Y. enterocolitica* (Ye) strains isolated from animals according to their source of isolation, origin and year of reception.

No. of strain	Bio-sero-phagetypes	No. of Strains/ Source	Origin [a]	Year of Reception
1	Ye1B/O:1,2,3/Xo	1/capybara feces	UNESP-Bot	1998
37	Ye 4/O:3/VIII	1/monkey spleen abscess	IB–SP	1974
		1/dog diarrheic feces	UFRJ-RJ	1980
		2/dog normal feces	UFRJ-RJ	1985
		1/capybara feces	UNESP- Bot	1998
		26/pig diarrheic feces	Sec.Agr.PR- PR; UEL-PR; Sec.Agr.RS- RS;. UNESP-Bot	1979,1980, 1985
		5/marmoset hepatic abscess	IB-SP	1968,1979
		1/rat feces	UNESP-Bot	1970
1	Ye 4/O:3/IXa	1/bovine diarrheic feces	Sec.Agri.PR-PR	1984
1	Ye 2/O:3/Xz	1/capybara feces	UNESP-Bot	1998
29	Ye1A/O:5/Xz	20/dog normal feces	UFRJ-RJ	1980
		9/dog diarrheic feces	UFRJ-RJ	1980
5	Ye2/O:5/Xz	5/dog normal feces	UFRJ-RJ	1980
1	Ye1B/O:6,31/Xz	1/dog normal feces	UFRJ-RJ	1980

[a] UNESP:Universidade Estadual Paulista; IB:Instituto de Biologia; UFRJ:Universidade Federal do Rio de Janeiro; UEL:Universidade Estadual de Londrina; Sec. Agr. PR.:Secretaria de Agricultura do Paraná; Sec. Agr. RS:Secretaria da Agricultura do Rio Grande do Sul; Bot:Botucatu.

Table 4 – Distribution of types of 73 *Y. enterocolitica* (Ye) strains isolated from food and 67 strains isolated from the environment according to their source of isolation, origin and year of reception.

No. of Strains	Bio-sero-phagetypes	No.of Strains/Source	Origin [a]	Year of Reception
Food				
2	Ye1A/O:4,33/Xz	2/meat	UFRJ-RJ	1984
20	Ye1A/O:5/Xz or Xz	16/milk	UFRJ-RJ; FCF-Ar.	1980, 1982, 1983
		1/meat	UFRJ-RJ	1984
		1/sausage	UNESP-Ar	1988
		2/meat	UFRJ-RJ	1984
11	Ye1A/O:5,27/Xz	5/ milk	UFRJ-RJ; FCF-SP	1980, 1991
		4/meat	UFRJ-RJ; USP-SP	1984, 1991
		1/sausage	ITAL-Camp.	1988
		1/ice	UNESP-Ar	1995
2	Ye 1A/O:6,31/Xz	1/milk	ITAL-Camp.	1983
		1/meat	UFRJ-RJ	1984
5	Ye 1A/O:7,8/Xo	1/milk	UFRJ-RJ	1980
		1/hamburger	FIOCRUZ-RJ	1998
		3/oyster	FIOCRUZ-RJ	1997
1	Ye 2/O:7,8/Xz	1/milk	UFRJ-RJ	1980
2	Ye1A/O:8,19/Xz	2/milk	FCF- SP	1988, 1991
3	Ye 1A/O:10/Xo or Xz	2/milk	UFRJ-RJ; FIOCRUZ- RJ	1984, 1997
		1/milk	FCF- SP	1991
8	Ye 2/O:10/Xo	8/milk	UFRJ-RJ; FIOCRUZ- RJ	1980, 1997
1	Ye1A/O:10,34/Xo	1/milk	FCF- SP	1991
1	Ye1A/O:12,25/Xo	1/meat	FCF- SP	1991
1	Ye1A/O:13/Xo	1/milk	UFRJ-RJ	1980
3	Ye A/O:13,7/Xz	2/meat	UFRJ-RJ; FCF-SP	1984, 1991
		1/vegetable	USP-SP	1991
5	Ye 1A/O:14/Xo	4/meat	UFRJ-RJ; FCF-SP	1984, 1991
		1/sausage	ITAL-Camp.	1988
1	Ye 2/O:14/Xz	1/milk	ITAL-Camp.	1982
1	Ye 2/O:36/Xo	1/milk	FIOCRUZ- RJ	1997
1	Ye 2/O:38/Xo	1/chicken pieces	FIOCRUZ- RJ	1998
4	Ye1A/O:41,42/Xo or	2/milk	FCF-SP	1991
		2/meat balls	FIOCRUZ- RJ	1998
1	Ye 1A/NAG/Xz	1/sausage	ITAL-Camp.	1988
Environment				
4	Ye 1A/O:5/Xz	2/polluted river	UFRJ-RJ	1983,1984
		2/waterfall	UFRJ-RJ	1983,1984
34	Ye 2/O:5,27/Xz	32/fresh water	CETESB-SP	1982
		2/sewage	CETESB-SP	1982
4	Ye 3/O:5,27/Xz	3/ocean	UFRJ-RJ	1984
		1/sewage	CETESB-SP	1982
1	Ye 1A/O:10/Xo	1/waterfall	UFRJ-RJ	1984
1	Ye 1A/O:16/Xz	1/ocean	UFRJ-RJ	1983
23	Ye 1A/O:27/Xz	23/sewage	CETESB- SP	1982

[a] FCF-SP:Faculdade de Ciências Farmacêuticas-USP; FCF-AR:Faculdade de Ciências Farmacêuticas-UNESP; UFRJ:Universidade Federal do Rio de Janeiro; UNESP:Universidade Estadual Paulista; USP:Universidade de São Paulo; ITAL:Instituto de Tecnologia de Alimentos; FIOCRUZ:Fundação Oswaldo Cruz; CETESB:Companhia de Tecnologia de Saneamento Ambiental; Ar.:Araraquara; Camp.:Campinas

Table 5 – Distribution of types of 67 *Y. intermedia* (Yi) strains isolated from humans (one), animals (two) and environment (64) according to their source of isolation, origin and year of reception.

No. of Strains	Bio-sero-phagetypes	No. of Strains/ Source	Origin [a]	Year of Reception
Human				
1	Yi 1/NAG/Xz	1/human diarrheic feces	UFRJ-RJ	1980
Animal				
1	Yi 1/O:12/Xo	1/capybara vaginal secretion	UNESP-Bot	1999
1	Yi 1/ O:17/Xz	1/dog diarrheic feces	UFRJ-RJ	1980
Environment				
3	Yi 1/O:4,32/Xz	1/fresh water	CETESB- SP	1982
		2/sewage	CETESB- SP	1982
9	Yi 4/O:4,32/Xz	9/sewage	CETESB- SP	1982
7	Yi 1/O:4,33/Xz	5/polluted river	UFRJ-RJ	1984
		2/polluted lagoon	UFRJ-RJ	1984
1	Yi 2/O:4,33/Xz	1/ocean	UFRJ-RJ	1984
1	Yi 4/O:7,8/Xz	1/fresh water	CETESB- SP	1982
6	Yi 1/O:10 Xz or Xo	2/ocean	UFRJ-RJ	1984
		2/polluted river	UFRJ-RJ	1984
		1/fresh water	CETESB- SP	1982
		1/waterfall	UFRJ-RJ	1984
1	Yi 1/O:13,7/Xo	1/fresh water	CETESB- SP	1984
7	Yi 1/O:14/Xo or Xz	1/fresh water	CETESB- SP	1982
		6/polluted river	UFRJ-RJ	1984
1	Yi 1/O:15/Xz	1/polluted river	UFRJ-RJ	1984
2	Yi 4/O:16/Xz	2/ocean	UFRJ-RJ	1984
1	Yi 1/O:17/Xz	1/polluted estuary	UFRJ-RJ	1984
1	Yi 1/O:18/Xz	1/polluted estuary	UFRJ-RJ	1984
2	Yi 1/O:25/Xo	1/fresh water	CETESB- SP	
		1/polluted river	UFRJ-RJ	1984
1	Yi 1/O:33/Xz	1/polluted estuary	UFRJ-RJ	1984
1	Yi 1/O:37/Xz	1/waterfall	UFRJ-RJ	
6	Yi 1/O:40/Xz or Xo	5/sewage	CETESB- SP	1982
		1/polluted river	UFRJ-RJ	1984
1	Yi 4/O:40/Xo	1/polluted river	UFRJ-RJ	
10	Yi 1/O:48/Xo or Xz	9/polluted river	UFRJ-RJ	1984
		1/polluted estuary	UFRJ-RJ	1984
1	Yi 1/O:66/Xz	1/polluted river	UFRJ-RJ	1984
2	Yi 1/NAG/Xz	1/estuary	UFRJ-RJ	1984
		1/polluted estuary	UFRJ-RJ	1984

[a] UFRJ:Universidade Federal do Rio de Janeiro; UNESP:Universidade Estadual Paulista; CETESB:Companhia de Tecnologia de Saneamento Ambienta de São Paulo.

Table 6 – Distribution of types of 132 *Y. intermedia* (Yi) strains isolated from food according to their source of isolation, origin and year of reception.

No. of Strains	Bio-sero-phagetypes	No. of Strains/ Source	Origin [a]	Year of Reception
1	Yi 1/O:3/Xz	1/milk	FCF-SP	1988
1	Yi 1/O:4/Xo	1/oyster	FIOCRUZ – RJ	1997
4	Yi 1/O:4,32/Xo or Xz	4/cheese	ITAL – Camp.	1984
1	Yi 1/O:4,33/Xo	1/oyster	FIOCRUZ – RJ	1998
2	Yi 1/O:5,27/Xz or Xo	1/milk	FCF – Ar.	1983
		1/meat	UFRJ – RJ	1984
1	Yi 3/O:5,27/Xz	1/meat	FCF – SP	1991
1	Yi 1/O:6,30,47/Xo	1/cheese	ITAL – Camp.	1984
3	Yi 1/O:7,8/Xo	3/milk	UFRJ – RJ	1980
1	Yi 1/O:10,34/Xo	1/meat	UFRJ – RJ	1984
1	Yi 3/O:10,34/Xo	1/meat	FCF – SP	1991
1	Yi 1/O:12,25/Xz	1/meat	FCF – Ar.	1984
14	Yi 1/O:13,7/Xz	13/meat	UFRJ – RJ	1984
		1/meat	FCF – Ar.	1984
1	Yi 3/O:13,7/Xo	1/meat	FCF – Ar.	1984
1	Yi 4/O:13,7/Xz	1/meat	UFRJ – RJ	1984
2	Yi 1/O:14/Xz	1/milk	ITAL – Camp.	1983
		1/bovine liver	FCF – Ar.	1984
2	Yi 6/O:14/Xz	2/milk	ITAL – Camp.	1983
1	Yi 8/O:14/Xo	1/milk	FCF – SP	1988
7	Yi 1/O:16/Xo or Xz	1/vegetable	FCF– Ar.	1984
		1/sausage	ITAL – Camp.	1988
		4/milk	FCF– SP	1988, 1990
		1/milk	FIOCRUZ - RJ	1997
1	Yi 4/O:16/Xo	1/milk	FCF – SP	1988
4	Yi 5/O:16/Xo	4/milk	UFRJ - RJ	1983
2	Yi 1/O:16,29/Xz	1/bovine liver	FCF – Ar.	1984
		1/meat	UFRJ - RJ	1984
1	Yi 1/O:16,58/Xo	1/meat balls	FIOCRUZ - RJ	1998
8	Yi 1/O:17/Xo	2/milk	FCF – SP	1991
		6/meat	UFRJ - RJ	1984
1	Yi 6/O:17/Xo	1/meat	UFRJ - RJ	1984
16	Yi 1/O:18/Xz or Xo	15/milk	UFRJ – RJ	1980
		1/milk	ITAL – Camp.	1981
1	Yi 1/O:21/Xz	1/meat	UFRJ - RJ	1984
1	Yi 1/O:22/Xo	1/milk	FCF – SP	1990
1	Yi 6/O:26/Xo	1/milk	ITAL – Camp.	1983
1	Yi 1/O:27/Xz	1/meat	FCF – Ar.	1984
1	Yi 1/O:29/Xz	1/meat	UFRJ - RJ	1984
2	Yi 1/O:33/Xo	1/milk	FCF – SP	1988
		1/sausage	ITAL – Camp.	1988
2	Yi 4/O:33/Xo	1/milk	FCF – SP	1990
		1/milk	UFRJ – RJ	1994
1	Yi 5/O:33/Xo	1/milk	FIOCRUZ - RJ	1997
1	Yi 1/O:33,37/Xz	1/milk	UFRJ - RJ	1980
1	Yi 1/O:36/Xo	1/meat	UFRJ - RJ	1984
2	Yi 1/O:37/Xo or Xz	1/hamburger	FCF – Ar.	1986
		1/milk	FCF – SP	1988
3	Yi 1/O:38/Xo	3/chicken pieces	FIOCRUZ - RJ	1998
3	Yi 1/O:40/Xo or Xz	1/raw milk	UFRJ – RJ	1980
		2/cheese	ITAL – Camp.	1980
1	Yi 2/O:40/Xo	1/milk	UFRJ - RJ	1980
10	Yi 4/O:40/Xo or Xz	2/cheese	UFRJ – RJ	1980
		3/milk	FIOCRUZ - RJ	1997
		5/cheese	ITAL – Camp.	1983
2	Yi 1/O:49/Xo	2/milk	FCF – SP	1990
5	Yi 1/O:52/Xz	4/milk	FCF – SP	1988, 1992
		1/meat	FCF – SP	1991
3	Yi 1/O:55/Xo	1/cheese	ITAL – Camp.	1984
		1/sausage	ITAL – Camp.	1988
		1/oyster	FIOCRUZ - RJ	1997
1	Yi 1/O:61/Xo	1/cheese	UFRJ - RJ	1988
2	Yi 1/O:65/Xo	1/meat	UFRJ – RJ	1984
		1/meat balls	FIOCRUZ - RJ	1998
1	Yi 1/O:66/Xz	1/milk	UFRJ - RJ	1980
2	Yi 1/O:69/Xz	2/milk	FCF– SP	1988
5	Yi 1/NAG/Xo or Xz	1/meat	UFRJ – RJ	1984
		1/oyster	FIOCRUZ – RJ	1998
		1/meat balls	FIOCRUZ – RJ	1998
		2/chicken pieces	FIOCRUZ - RJ	1998
1	Yi 4/NAG/Xo	1/milk	FCF – SP	1990
1	Yi 5/NAG/Xo	1/meat	FCF – SP	1991

[a]FCF-SP:Faculdade de Ciências Farmacêuticas-USP, FCF-Ar:Faculdade de Ciências Farmacêuticas-UNESP; USP:Universidade de São Paulo; UNESP:Universidade Estadual Paulista; FIOCRUZ:Fundação Oswaldo Cruz; ITAL:Instituto de Tecnologia de Alimentos; UFRJ:Universidade Federal do Rio de Janeiro; Camp.:Campinas; Ar.:Araraquara.

Table 7 - Distribution of types of 87 *Y. frederiksenii* (Yf) isolated from humans (two), animals (five), food (70) and environment (10), according to their source of isolation, origin and year of reception.

No. of Strains	Sero-phagetypes	No. of Strains/ Source of Isolation	Origin [a]	Year of Reception
Human				
1	Yf O:16a,58/Xo	1/diarrheic feces	IAL-RP	1997
1	Yf O:74/Xo	1/diarrheic feces	IAL-RP	1993
Animal				
4	Yf O:16/Xo	4/dog	UFRJ-RJ	1980
1	Yf O:16Xo	1/pig diarrheic feces	UEL- PR	1985
Food				
1	Yf O:2,3/Xz	1/meat	UFRJ-RJ	1984
4	Yf O:10/Xo	4/milk	USP-SP	1988
1	Yf O:10,34/Xo	1/meat	USP-SP	1991
7	Yf O:12,25/Xz	7/milk	UFRJ-RJ	1983
1	Yf O:13,7/Xz	1/vegetable	USP-SP	1991
14	Yf O:16/Xo	3/milk	USP-SP	1991
		11/cheese	UFRJ-RJ	1988
1	Yf O:16,29/Xz	1/meat	UFRJ-RJ	1984
2	Yf O:16a,58/Xo	1/vegetable	USP-SP	1991
		1/hamburger	FIOCRUZ-RJ	1998
2	Yf O:22/Xz	1/milk	UFRJ-RJ	1980
		1/vegetable	USP-SP	1991
1	Yf O:25/Xz	1/milk	UFRJ-RJ	1983
2	Yf O:27/Xo orXz	2/milk	UFRJ-RJ	1983
2	Yf O:38/XorXz	1/meat balls	FIOCRUZ-RJ	1998
		1/chicken pieces	FIOCRUZ-RJ	1998
19	Yf O:40/Xo orXz	18/milk	UFRJ-RJ	1983, 1984
		1/sausage	ITAL-Camp.	1980
1	Yf O:40,74/Xo	1/oyster	FIOCRUZ-RJ	1997
1	Yf O:52/Xz	1/sausage	ITAL-Camp.	1988
2	Yf O:60/Xo	1/meat	USP-SP	1991
		1/chicken pieces	FIOCRUZ-RJ	1998
1	Yf O:66/Xz	1/cheese	ITAL-Camp.	1984
1	Yf O:74/Xo	1/sausage	ITAL-Camp.	1988
7	Yf NAG/Xo or Xz	1/milk	USP-SP	1990
		2/oyster	FIOCRUZ-RJ	1997
		2/chicken pieces	FIOCRUZ-RJ	1998
		2/meat	USP-SP	1991
Environment				
1	Yf O:2,3 Xz	1/polluted lagoon	UFRJ-RJ	1984
3	Yf O:10 Xo or Xz	2/stream	UFRJ-RJ	1984
		1/spring	UFRJ-RJ	1984
6	Yf O:16 Xo or Xz	5/spring	UFRJ-RJ	1984
		1/polluted estuary	UFRJ-RJ	1984

[a] IAL:Instituto Adolfo Lutz; UFRJ:Universidade Federal do Rio de Janeiro; FIOCRUZ:Fundação Oswaldo Cruz; ITAL:Instituto de Tecnologia de Alimentos; USP:Universidade de São Paulo; Camp.:Campinas; RP:Ribeirão Preto. UEL:Universidade de Londrina

*(*46.2%)*, Y. intermedi* (44.2%), *Y. kristensenii* (2.0%), *Y. frederiksenii*(7.6%) and one Non-tipable. The environmental *Y.enterocolitica* strains belong to pathogenic and non-pathogenic bio-serotypes.

The Non-typable (atypical) strains were isolated from food and water. Perhaps, some of these strains may be classified as a new *Yersinia* species. Studies on the genetic relatedness among those atypical strains and the 12 known and defined *Yersinia* species have been performed by our group.

Early reports on the occurrence of some virulence markers in *Y. enterocolitica* strains isolated in Brazil were made by Nunes & Ricciadi (1981). These investigators searched for the presence of enterotoxin, using the suckling mouse bioassay. Toledo et al. (1982) searched for the production of LT and ST enterotoxins and adherence factors and for the ability to invade guinea pig eye (by using the Sereny test), in some strains of *Y. enterocolitica* of the following bio-serotypes: 4/O:3, 1A/O:5 and 2/O:5. Similarly, Nunes et al. (1985) tested *Y.enterocolitica* and *Yersinia* strains of other species isolated from human and dogs in Rio de Janeiro-RJ, for invasion and enterotoxin production. Pestana de Castro et al. (1983), tested *Y. enterocolitica* strains, isolated from pigs, for the production of ST and LT enterotoxins, presence of adhesins and invasive ability using the Sereny test.

Falcão et al. (1984) and Bauab & Falcão (1991) studied the *in vivo* pathogenicity of strains of *Y. enterocolitica* bio-serotypes: 2/O:8, 4/O:3, 2/O:5, 1A/O:5, 1A/O:4,32 and *Y. intermedia, Y. frederiksenii* and *Y. kristensenii* isolated from human and animal clinical material and from food, by experimental infection and monitoring growth kinetics in mice after intragastric and intravenous inoculation. In the first study (Falcão et al.,1984), it was shown that the strains isolated from human and animal clinical cases, of biotypes and serotypes related to disease, invaded and multiplied in different organs and tissues, whereas the strains isolated from food and patients without clinical symptoms were only detected in the cecal content. However it was not verified if this difference in the severity of infection was due to the presence or the absence of virulence markers in the strains of different *Yersinia* species isolated from various sources.

Table 8 - Distribution of 13 *Y. kristensenii* (Yk) strains isolated from animals (one), food (nine) and environment (three), according to their source of isolation, origin and year of reception

No. of Strains	Sero-phagetypes	Number of Strains/Source	Origin [a]	Year of Reception
Animal				
1	Yk NAG/ Xo	1/capybara feces	UNESP-Bot.	1998
Food				
2	Yk O: 9/ Xo or Xz	2/ milk	ITAL-Camp.	1981
2	Yk O:11/ Xo or Xz	1/ meat	UFRJ- RJ	1984
		1/ sausage	ITAL-Camp.	1988
2	Yk O:11,24/Xo or Xz	2/ meat	UFRJ- RJ	1984
2	Yk O: 61/ Xo or Xz	1/ meat	UFRJ- RJ	1984
		1/ oyster	FIOCRUZ-RJ	1998
1	Yk NAG/ Xz	1/ meat	UFRJ- RJ	1984
Environment				
1	Yk O: 11,24/ Xo	1/ polluted river	UFRJ- RJ	1984
2	Yk O: 61/ Xo or Xz	1/ waterfall	UFRJ- RJ	1984
		1/ polluted estuary	UFRJ- RJ	1984

[a] UNESP:Universidade Estadual Paulista; ITAL:Instituto de Tecnologia de Alimentos; UFRJ:Universidade Federal do Rio de Janeiro; FIOCRUZ:Fundação Oswaldo Cruz; Bot.:Botucatu; Camp.:Campinas.

Table 9 – Distribution of types of 105 *Y. pseudotuberculosis* (Yp) strains isolated from animals according to their source of isolation, origin and year of reception

No. of Strains	Bio-sero-types	No. of Strains/ Source	Origin [a]	Year of Reception
3	Yp 1/O:3	3/buffalo mesenteric ganglion	UFPEL-RS	1989
102	Yp 2/O:3	54/bovine diarrheic feces	CDME-PR	1984,1985,1987, 1989,1990
			UEL-PR	1985
		2/bovine intestinal loop	CDME-PR	1983
		9/bovine small intestine material	CDME-PR	1985,1989,1990
		5/bovine large intestine material	CDME-PR	1989,1990
		5/buffalo diarrheic feces	UFPEL-RS	1989,1990
		25/buffalo normal feces	UFPEL-RS	1990
		1/buffalo organ	UFPEL-RS	1982
		1/pig diarrheic feces	UEL-PR	1985

[a] UFPEL:Universidade Federal de Pelotas-RS; CDME:Centro de Diagnóstico Marcos Enrietti de Curitiba-PR; UEL:Universidade Estadual de Londrina-PR.

Table 10 - Distribution of six atypical *Yersinia* (NT)[b] strains isolated from food (five) and environment (one), according to their source of isolation, origin and year of reception.

No. of Strains	Sero-phagetypes	Number of Strains/Source	Origin [a]	Year of reception
Food				
1	NT O:10,34/Xo	1/vegetable	FCF-SP	1991
1	NT O: 12,25/Xo	1/milk	FCF-SP	1991
1	NT O: 22/Xz	1/milk	FCF-SP	1990
2	NT O:25/Xz	2/ground meat	FCF-Ar.	1984
Environment				
1	NT O:10/Xz	spring	UFRJ- RJ	1984

[b] NT: non-typable;
[a] FCF-SP:Faculdade de Ciências Farmacêuticas–USP; FCF-Ar.:Faculdade de Ciências Farmacêuticas-UNESP;.
UFRJ: Universidade Federal do Rio de Janeiro

As a continuation of the study just mentioned above, Bauab and Falcão (1991) inoculated mice intragastrically and intravenously with the same *Yersinia* strains, bearing or not bearing the virulence plasmid and other chromosomal virulence markers. The results led us to conclude that it was possibly a invasin or several invasins of chromosomal origin that permitted the pathogenic strains to invade various organs and tissues of the animals and to multiply regardless of the presence of the virulence plasmid; but this plasmid determined the intensity of the clinical symptoms.

Perroni et al. (1995) studied 46 strains of *Y. enterocolitica* 4/O:3 isolated from human clinical material and found the virulence plasmid pYV in all of them. Furthermore, they showed that these isolates exhibited the phenotypic markers expressed by the plasmid virulence genes.

Falcão et al. (2003) reported that strains of *Y. enterocolitica* 4/O:3, isolated from sick humans and animals, in different regions of Brazil, exhibited virulence genes detected by PCR and virulence phenotypic markers, as well as, multiple drug-resistance profiles, confirming their pathogenic potential.

Martins et al. (1998) and Martins & Falcão (2003), using phenotypic tests and PCR, searched for virulence markers in 105 *Y. pseudotuberculosis* strains , isolated from animals in the states of Paraná and Rio Grande do Sul and found that all of them had some or all of the virulence markers tested. Also Martins & Falcão (2004) assayed the *in vivo* kinetics of infection of some virulent strains of *Y. pseudotuberculosis* in a mouse model, finding that those strains harboring the pYV plasmid and with all the genes of the high pathogenicity island (HPI) were more invasive *in vivo* than those without the plasmid or the HPI genes. Additionaly, Martins et al. (2007) performed ribotyping to determine the genetic similarity among the *Y. pseudotuberculosis* isolates. The strains showed a high level of similarity among each other, just four main ribotypes being observed overall.

Warnken et al. (2001; 2002), using the RAPD-PCR technique in a preliminary study of Brazilian *Yersinia* strains isolated from several kinds of food in Rio de Janeiro, observed a low level of polymorphism in this species, allowing strains to be differentiated according to their origin.

Studies made by Falcão (2004) and Falcão et al. (2006) showed that two strains of *Y. enterocolitica* out of a total of 35 isolated from food possessed virulence genes. Most of the *Y. enterocolitica* food strains studied were of bio-serotype 1A/O:5 and 1A/O:5,27, while those carrying the virulence genes were of bio-serotypes 1A/O:10 and 1A/O:6,30. Such results add to the growing evidence, published in recent studies, that the biotype 1A is probably not as innocuous as it was suggested in the past (Tennant et al., 2003).

Falcão et al. (2004) studied all the 145 isolates from the environment that belong to the National Reference Center on *Yersinia* spp in Brazil, with regard to their virulence potential using molecular and phenotypic tests. These strains gave variable results as follow: virulence markers were found in all strains of *Y.enterocolitica* of bio-serotypes 2/O:5,27 and 3/O:5,27; but not in any strain of *Y. enterocolitica* biotype 1A of serogroups O:5, O5,27, O:10, O:16 and O:27. Similarly, no virulence markers were found in any the of *Y.intermedia, Y.kristensenii and Y.frederiksenii* strains isolated from the environment.

These data regarding *Yersinia* in Brazil summarize our findings at the National Reference Center on *Yersinia* spp. other than *Y. pestis*. However, we believe that there are other *Yersinia* cultures isolated in Brazil, which have not been delivered to the Reference Laboratory and consequently were not included in this paper.

RESUMO

Panorama geral da ocorrência de espécies de Yersinia *que não* Yersinia pestis *no Brasil*

Dados sobre a ocorrência de espécies de *Yersinia* que não a *Y. pestis* no Brasil são apresentados. Nos últimos 40 anos, 767 linhagens de *Yersinia* foram identificadas e tipadas pelo Laboratório Nacional de Referência em *Yersinia* spp. outras que *Y. pestis*, usando testes bioquímicos clássicos para a caracterização das espécies. As linhagens foram posteriormente classificadas em biotipos, sorotipos e fagotipos quando pertinente. Estes testes possibilitaram a identificação de culturas de *Yersinia*, pertencentes às espécies: *Y. enterocolitica, Y. pseudotuberculosis, Y. intermedia, Y. frederiksenii* e *Y. kristesenii*. Seis isolados que não puderam ser classificados bioquimicamente em alguma das espécies de *Yersinia* conhecidas foram denominadas de não-tipáveis (NT). Essas linhagens foram tipadas em diversos bio-soro-fagotipos. Não foram identificadas pelos testes bioquímicos convencionais representantes das espécies *Y. aldovae, Y. rhodei, Y. mollaretii, Y. bercovieri* e *Y. ruckeri*. As linhagens de *Yersinia* foram isoladas de materiais clínicos de humanos e animais sadios e/ou doentes, de diferentes tipos de alimentos e do meio ambiente, por pesquisadores de várias Instituições localizadas em diferentes cidades e regiões do Brasil.

Palavras-chave: *Yersinia* spp.; ocorrência; Brasil

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