Frequency of *Yersinia* species infection in paediatric acute diarrhoea in Tehran

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تواتر العدوى بأنواع اليرْسَنيّات في الإسهال الحاد لدى الأطفال في طهران

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الخلاصة: توضح هذه الدراسة تواتر العدوى باليرْسَنيّات المُمْلِهة للمعي والقولون لدى 300 طفلاً أصيبوا بالإسهال الحاد وتقل أعمارهم عن 12 عاماً، ممن راجعوا مستشفى الأطفال في طهران. واستمرت الدراسة خمسة أشهر (من أيار/مايو إلى أيلول/سبتمبر 2002)، وتم خلالها إجراء الزرع لأنواع اليرْسَنيّات ولغيرها من العوامل الممرضة، مع تعيين النمط السيرولوجي من المبيتات أو المسحات الوازية. وقد أمكن كشف أنواع اليرْسَنيّات في 8 حالات (2.7٪) والإيشريكية القولونية الممرضة للأمعاء في 5.7٪ من الحالات وأنواع الشيغيلات في 3٪ من الحالات وأنواع السالمونيلات في 2٪ من الحالات. ولم تكشف أيٌّ حالة من اليرْسَنيّات المُمْلِهة للمعي والقولون التي تنضوي تحت النمطين السيرولوجيين الشائعين O:9 وO:3. في حين تم استفراد بعض الأنواع اللاّنموذجية من اليرْسَنيّات (اليرْسَنيّات المتوسطة واليرْسَنيّات الفريدريكسونية). وقد أبدَتْ جميع مستفردات اليرْسَنيّات غراراً متشابهاً من حيث المقاومة لمضادات المكروبات. على أن العدوى بأنواع اليرْسَنيّات غير شائع في أشهر الصيف في طهران.

ABSTRACT This study determined the frequency of *Yersinia enterocolitica* infection in 300 children with acute diarrhoea aged 0–12 years who were attending a paediatric hospital in Tehran. Over the 5-month study (May–September 2002), *Yersinia* species and other organisms were cultured and serotyped from stool samples or swabs. *Yersinia* spp. were found in 8 cases (2.7%). Enteropathogenic *Escherichia coli* was isolated in 5.7% of cases, *Shigella* spp. in 3.0% and *Salmonella* spp. in 2.0%. None of the *Y. enterocolitica* belonged to the common serotypes of O:3 and O:9. Atypical *Yersinia* spp. (*Y. intermedia* and *Y. frederiksenii*) were isolated. All *Y. enterocolitica* isolates had a similar pattern of antimicrobial resistance. *Yersinia* spp. infections are not common in the summer months in Tehran.

Fréquence de l'infection par les espèces de *Yersinia* dans la diarrhée aiguë de l'enfant à Téhéran

RESUME Cette étude a déterminé la fréquence de l'infection à *Y. enterocolitica* chez 300 enfants âgés de 0 à12 ans souffrant de diarrhée aiguë qui consultaient dans un hôpital pédiatrique de Téhéran. Pendant l'étude qui a duré cinq mois (mai-septembre 2002), les espèces de *Yersinia* et d'autres organismes ont été mis en culture et sérotypés à partir d'échantillons fécaux ou de prélèvements effectués à l'aide d'un écouvillon. On a trouvé *Yersinia* spp dans 8 cas (2,7 %). *Escherichia coli* entéropathogène a été isolé dans 5,7 % des cas, *Shigella* spp dans 3,0 %, et *Salmonella* spp dans 2,0 %. Aucune des *Y. enterocolitica* spp n'appartenait aux sérotypes courants O:3 et O:9. Des espèces atypiques du genre *Yersinia* (*Y. intermedia* et *Y. frederiksenii*) ont été isolées. Tous les isolats de *Y. enterocolitica* avaient un profil de résistance antimicrobienne similaire. Les infections à *Yersinia* spp ne sont pas fréquentes durant les mois d'été à Téhéran.

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Introduction

Diarrhoeal diseases are a major cause of childhood morbidity and mortality worldwide, especially in developing countries [1]. They account for an estimated annual 5 million deaths among infants aged under 5 years around the world [2].

Yersinia enterocolitica is a pathogen that causes self-limiting gastroenteritis or enterocolitis [3,4]. The organism is particularly common among children, causing outbreaks in day-care centres and schools. Symptoms range from mild (diarrhoea, abdominal pain) to severe (fever, severe abdominal pain often mistaken for appendicitis). Occasionally *Y. enterocolitica* gastrointestinal infection is followed by arthritis of the peripheral joints [5,6]. In different parts of the world, such as southern and western Europe (Scandinavia, Belgium, Holland, France, Germany, Denmark, etc), the United States of America, Canada, Australia, Japan and many other countries, *Y. enterocolitica* has been shown to be a primary human pathogen [4,7–9].

Although *Yersinia* species (spp.) have been reported in tropical areas [10], infections are more prevalent in cold European countries and North America [11,12]. Since the first isolation of *Y. enterocolitica* in the Islamic Republic of Iran in 1977 [13], there have been only a few studies on the epidemiology of this pathogen in our country and only one study has reported its isolation from drinking-water in Tehran [14,15]. *Y. enterocolitica* has been isolated from humans in many countries with varying rates [6,16]. Infrequent isolation in some areas has led some investigators to conclude that routine culturing of stool specimens for *Yersinia* spp. is not cost-effective [17]. The current study aimed to record the frequency and serotypes of *Yersinia* spp. and other enteropathogens in children with acute diarrhoea attending a paediatric hospital in Tehran. We focused our study on children as they are two to three times more susceptible to infection with *Yersinia* spp. than adults.

Methods

Subjects

The subjects were 300 children between the ages of 0 and 12 years who were suffering from acute diarrhoea (3 or more loose or watery stools per day for a period of less than 2 weeks). We included all patients with a diagnosis of acute diarrhoea visiting the Children's Treatment Centre at Markaz Tebbi Koodakan during a 5-month period from May to September 2002. Markaz Tebbi Koodakan is the largest paediatric hospital in Tehran, the capital city of the Islamic Republic of Iran

Data collection

All the patients' demographic and clinical data were collected by the primary care physicians at the centre using a questionnaire. Stool samples were collected from the children or, when this was not possible, rectal swabs were taken. For the detection of *Yersinia* spp., the samples were transported to the laboratory in phosphate-buffered saline transport medium (pH 7.0), according to World Health Organization recommendations [18] (0.5–1 g stool in 5 mL of buffer). To isolate *Escherichia coli*, *Salmonella* spp. and *Shigella* spp., an additional swab was obtained from the stool sample or directly from the patient and was placed in Cary-Blair transport medium.

Laboratory analysis

For the detection of *Yersinia* spp. the cold enrichment method was used. Samples were incubated at 4 °C for 4 weeks. At the end of the first, second, third and fourth week of incubation, samples were then cul-

tured on *Yersinia*-selective agar (CIN, 1.16434.0500, Merck, Darmstadt, Germany) with *Yersinia*-selective supplement (CIN, 1.16466.0001, Merck) and on MacConkey agar (CIN, 1.05465.0500/5000, Merck, Darmstadt, Germany) and incubated at 22 °C for 24 and 48 hours. After 24-hour incubation on *Yersinia*-selective agar, the samples were inspected carefully for suspicious colonies: pinpoint, round pink to red colonies, with a clear colourless surrounding halo which was clearly visible after 48 hours. After 24 hours on Mac Conkey agar, tiny, round and colourless (lactose-negative) colonies were considered suspicious for *Y. enterocolitica*. Because of the lack of necessary anti-sera at the laboratory, the isolates were sent to the Pasteur Institute of Paris for serotyping.

To identify other organisms, the samples were transferred immediately to culture media. *E. coli* were cultured on Endo agar medium (CIN, 1.04044.0500/5000, Merck, Darmstadt, Germany) and suspicious colonies (red, sometimes metallic colonies) were transferred to differential media (Kligler iron agar, SIM medium, urea agar and Simmons citrate agar) and incubated for 18–24 hours at 37 °C. Identification of enteropathogenic *E. coli* was done by slide agglutination with commercial polyvalent antisera (BioMericux, Lyon, France). For isolation of *Salmonella* spp. and *Shigella* spp. samples were transferred to Salmonella Shigella (SS) agar (CIN, 1.07667.0500, Merck, Darmstadt, Germany). Selenite-F broth (CIN, BO0354, Oxoid, UK) was used to augment the isolation of *Salmonella* spp. Suspicious colonies for *Shigella* sp. (colourless colonies) and for *Salmonella* spp. (colourless sometimes with a black precipitate) were transferred to differential media and incubated as before. *Salmonella* and *Shigella* spp. were identified through standard techniques [*19*] and were seroptyped using commercially available antisera (Difco, Detroit, Michigan, USA). Stool were examined for ova and parasites using the formalin ethyl acetate concentration method.

The *in vitro* susceptibilities to a range of antibiotics were determined for all isolates by the disk diffusion method [*20*]. The antibiotics tested were: tetracycline, chloramphenicol, gentamicin, kanamycin, streptomycin, amikacin, colistin, polymyxin B, co-trimoxazole, nitrofurantoin, nalidixic acid, lincomycin, penicillin G, ampi- cillin, cephalothin and rifampicin.

Results

A total of 300 children were studied: 122 females (40.7%), 178 males (59.3%). The mean age ± standard deviation was 3.34 ± 3.21 years. Table 1 shows the age and sex distribution of the children. Most of the patients were younger than 1 year old, followed by those aged 1–3 years.

Enteropathogenic *E. coli* was the most common pathogen isolated (17 out of 300 cases, 5.7%). *Shigella* spp. were isolated from 9 cases (3.0%), *Yersinia* spp. from 8 (2.7%) and *Salmonella* spp. from 6 (2.0%) (Table 2). More than one pathogen was isolated from 2 children, who were positive

Table 1 Age and sex distribution of 300 children with acute diarrhoea

Age (years)	Male No.	Female No.	Total No.	Total %
< 1	63	47	110	36.7
1–3	46	33	79	26.3
4–6	31	22	53	17.7
7–9	28	14	42	14.0
10–12	10	6	16	5.3
Total	178	122	300	100.0

for both enteropathogenic *E. coli* and *Shigella* spp.

The most common serotypes of the isolated *E. coli* were O26:B6 (8 cases, 47.1%) and O119:B14 (3 cases, 17.7%). Among the *Shigella* spp., *Sh. flexneri* type 2 (4 cases, 44.4%) and *Sh. sonnei* (2 cases, 22.2%) were most common. In cases of *Salmonella* spp., the two serotypes isolated were *S. typhimurium* (5 cases, 83.3%) and *S. havana* (1 case, 16.7%).

Among the 8 *Yersinia* spp. isolates, 5 cases (62.5%) were *Y. enterocolitica*. The results of the serotyping for isolated *Y. enterocolitica* showed that none of them belonged to the common serotypes of O:3 and O:9, and they were all environmental serotypes. Furthermore, the atypical *Yersinia* species *Y. intermedia* or *Y. frederiksenii* were isolated from the other 3 cases (Table 3).

All of our *Y. enterocolitica* isolates had a similar pattern of antimicrobial resistance (Table 4). The *E. coli* were all were sensitive to nalidixic acid and colistin, while the pattern of resistance for the rest of the antibiotics differed for each strain. Among the *Salmonella* and *Shigella* spp., all were sensitive to nalidixic acid, colistin and amikacin and were resistant to the other antibiotics.

Among the cases with *Y. enterocolitica*, 60% (3 out of 5) lived in rural areas in Tehran province where the drinking-water supply is from wells; the rest were living in urban areas. Diarrhoea and abdominal pain and fever (38–39 °C) were the most common clinical manifestations of infection with *Yersinia* sp. and were observed in all cases. Vomiting, headache and anorexia were seen less frequently. Diarrhoea was sometimes accompanied by mucous but no blood was found in stools.

All of the 17 patients with enteropathogenic *E. coli* had a negative direct smear for parasites, except 1 patient who was positive for *Hymenolepis nana* and had a past medical history of long-term weakness, malaise and chronic diarrhoea. Eight (47.1%) of the enteropathogenic *E. coli* cases were less than 1 year old.

Table 2 **Distribution of isolated pathogens by sex from 300 children with acute diarrhoea**

Species	Male No.	Female No.	Total No.	%
Enteropathogenic *E.coli*	10	7	17	5.7
Shigella spp.	5	4	9	3.0
Yersinia spp.	5	3	8	2.7
Salmonella spp.	3	3	6	2.0

Table 3 **Biotypes and serotypes of *Yersinia* species isolated from 8 cases of acute diarrhoea**

Case no.	Species	Biotype	Serotypes
1	*Y. enterocolitica*	1	O:7, O:8, O:19
2	*Y. enterocolitica*	1	O:7, O:8, O:19
3	*Y. enterocolitica*	1B	Autoagglutinable
4	*Y. enterocolitica*	1	Non autoagglutinable
5	*Y. enterocolitica*	1	Non-autoagglutinable
6	*Y. frederiksenii*	–	O:39
7	*Y. intermedia*	2	O:17
8	*Y. intermedia*	1	O:17

Discussion

The main objective of our study was to isolate *Yersinia* spp. to find out whether this organism and its common serotypes exist in the Islamic Republic of Iran or not. We were able to isolate *Yersinia* spp. from 8 of our patients in whom the other enteric bac-

Table 4 **Antimicrobial susceptibility of *Yersinia* species isolated from 8 cases of acute diarrhoea**

Antibiotic	Sensitive		Resistant	
	No.	%	No.	%
Tetracycline	8	100	0	0
Chloramphenicol	8	100	0	0
Gentamicin	8	100	0	0
Kanamycin	8	100	0	0
Streptomycin	8	100	0	0
Amikacin	8	100	0	0
Colistin	8	100	0	0
Polymyxin B	8	100	0	0
Co-trimoxazole[a]	8	100	0	0
Nitrofurantoin	8	100	0	0
Nalidixic acid	8	100	0	0
Lincomycin	0	0	8	100
Penicillin G	0	0	8	100
Ampicillin	0	0	8	100
Cephalothin	0	0	8	100
Rifampicin	0	0	8	100

[a] *Sulfamethoxazole/trimethoprim.*

terial pathogens mentioned were excluded. Our findings clearly show that *Y. enterocolitica* is present as a pathogen of diarrhoea in this country and can be isolated from the stool samples of children suffering from acute diarrhoea. We found a frequency of 2.7% for this organism, which is near the frequency of about 1% reported by other studies in this country [*14*] but is lower than some parts of the world especially northern European countries with a frequency up to 13% [*7,11*]. This might be partly due to the warmer climate in our country, especially as the study was carried out during summer, and partly due to different dietary patterns in the Islamic Republic of Iran where pork is not consumed. We would expect a higher frequency during autumn and winter, based on the fact that this organism increases greatly in comparison with other species in cold seasons [*4,16*].

We used CIN medium and the cold enrichment method, which has been shown by many researchers to be an effective method for the isolation of *Yersinia* spp. from stool samples [*21–23*]. The *Y. enterocolitica* isolated were considered to be the cause of the presentation of acute diarrhoea, despite the fact that none of them belonged to the group of serotypes that are dominant in Europe, Asia, and Canada (O:3 and O:9) [*5,6*].

According to previous studies, the highest frequency of *Y. enterocolitica* is in cool-weather rural areas, based on the presence of the most important sources of contamination: pigs, cows, rabbits, and dogs and the surfaces and drinking-water sources contaminated with their faeces [*24–27*]. Our findings are in harmony with the previous studies since 3 out of 5 of our *Y. enterocolitica* cases lived in rural areas with a cooler climate and probably used contaminated drinking-water. This might also be the reason why all the isolated species belonged to the groups whose pathogenicity has been reported [*25,28–30*]. The main risk factors for the morbidity and mortality of diarrhoea are well known and relate to a poor quality of life, lack of sanitation and clean water supply for most of the population living in poor areas of developing countries [*29,30*].

The clinical manifestations of *Y. enterocolitica* infection in our children were mild, in accordance with studies from northern Europe [*5*] and in contrast with the study of Naqvi et al. [*4*]. Six of them had fever but we did not find any patients with bloody stools. The results of the antimicrobial sensitivity tests for *Yersinia* spp. iso-

lates showed a similar pattern to other studies [4,5].

Overall, enteropathogenic *E. coli* was the most common isolated pathogen in our study (5.7%), and this is consistent with other studies in the Islamic Republic of Iran [14] and in other developing countries [31]. The findings for *Salmonella* and *Shigella* spp. isolates were also consistent with these studies [14,31].

In spite of the fact that *Y. enterocolitica* is an important cause of diarrhoea in some European and Scandinavian countries with a colder climate, this study has shown that this organism is rarely isolated from stool cultures of children with diarrhoea seen in a hospital setting in the summer months in Tehran.

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