

## Occurrence of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in Rodents and Cat Feces from Riyadh Area, Saudi Arabia

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**ABSTRACT.** *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* were isolated from 83 (20.4%) out of 406 rodents and from 64 (8.8%) out of 725 cat feces samples. The isolates from rodents include 59 *Y. enterocolitica* and 24 *Y. pseudotuberculosis*, whereas, the isolates from cat feces include 19 *Y. enterocolitica* and 45 *Y. pseudotuberculosis*. The rates of virulent *Y. enterocolitica* and *Y. pseudotuberculosis* in rodent isolates were found to be 10.2% and 79.2% respectively, whereas, for cat fecal isolates these rates were 42.1% and 86.7%, respectively. The highest incidence of the two species in both cat feces and rodents occurred in the cold months.

*Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are the causative agents of human gastroenteritis and terminal ileitis. The incidence of human infections by both agents has increased dramatically in recent years (Clover and Abner 1989, Fukushima *et al.* 1987, 1988, 1989, Metchock *et al.* 1991). Since the infections have been considered as zoonoses, some investigators have attempted their isolation from various species of animals and found that they are widely distributed among domestic pets (Fukushima *et al.* 1984, Salamah and Makki 1991, Yanagawa *et al.* 1978), farm animals (Fukushima *et al.* 1983, Shiozawa *et al.* 1988, Slee and Skilbeck 1992, Tsubokura *et al.* 1984), and wild animals (Fukushima *et al.* 1990, Kaneko and Hashimoto 1981, Kato *et al.* 1985, Mair 1973, Shayegani *et al.* 1986).

Recently *Y. enterocolitica* was isolated from sewage water, irrigation water, and chicken fecal contents (Salamah and Makki 1991). The present study was

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**Running Title :** Occurrence of *Yersinia* spp. in Saudi Arabia.

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undertaken to determine the incidence of *Y. enterocolitica* and *Y. pseudotuberculosis* in cats and rodents from the Riyadh area, Saudi Arabia.

### Materials and Methods

#### Samples

From December 1989 to March 1990, 406 rodents were trapped and 725 cat feces samples were collected from 9 regions in Riyadh, Saudi Arabia (Fig.1). The rodents

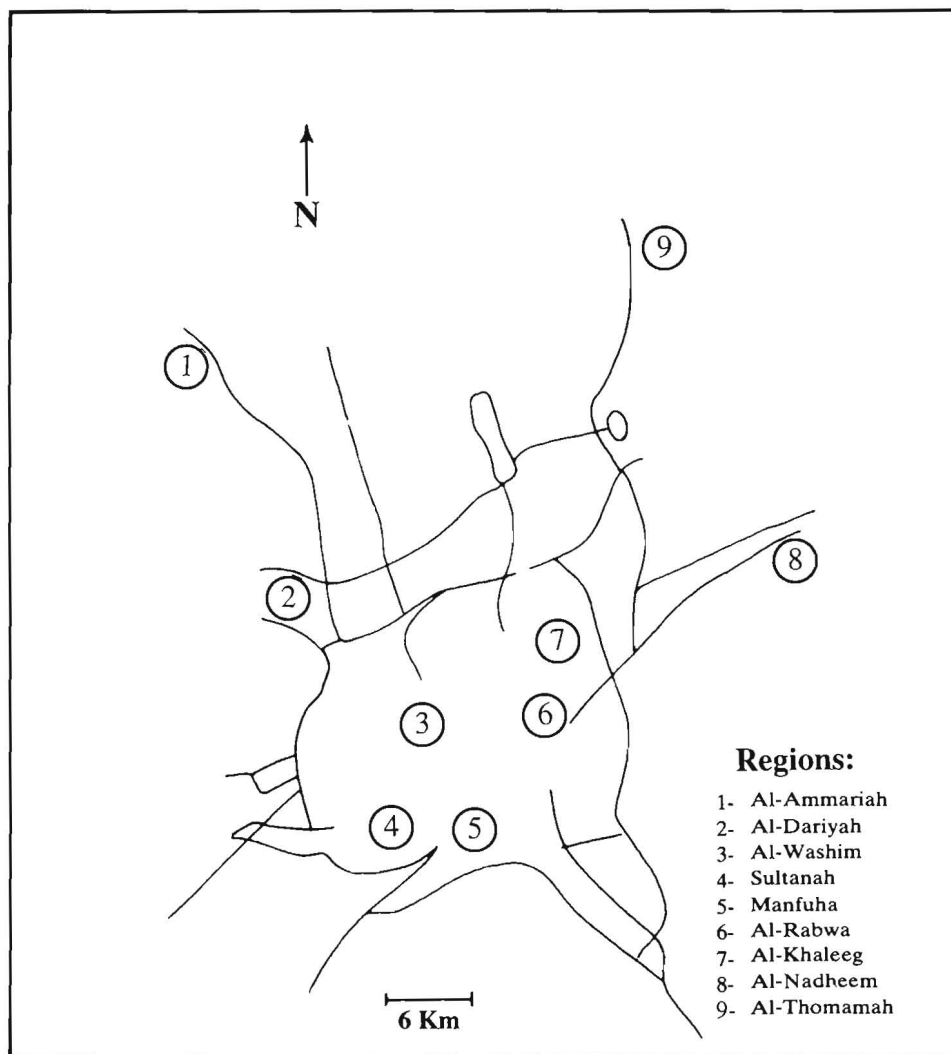


Fig. 1. Sketch map of Riyadh area (Saudi Arabia) showing regions from which samples were collected.

include *Rattus rattus*, *Gerbillus gerbillus*, *Meriones crassus* and *Meriones libycus*. The animals were sacrificed by CO<sub>2</sub> suffocation within 12 h of trapping. Cat feces was collected using sterile forceps and sterile Petri dishes and processed within 2-3 h of their collection.

The seasonal incidence of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in *R. rattus* and in cat feces was studied in the Manfuha region throughout 1991.

#### *Isolation and identification of Yersinia spp.*

Approximately 0.1 g of the fecal contents of each rodent or 0.1 g of each cat feces sample was suspended in 10 ml of 0.067 M phosphate buffer solution (pH 7.6) and kept at 4°C for 21 days. *Y. enterocolitica* and *Y. pseudotuberculosis* are able to grow at 4°C with an optimal incubation time of 21 days, whereas other enteric bacteria fail to grow or even decline in number. The suspensions were then subcultured on *Yersinia* selective agar (Merck) and MacConkey agar (Oxoid) with KOH treatment following enrichment (Aulisio *et al.* 1984). Colonies morphologically similar to *Yersinia* spp. were subcultured and identified as described by Kato *et al.* (1985).

#### *Assay of virulence-associated properties.*

All isolates identified as *Y. enterocolitica* or *Y. pseudotuberculosis* were examined for calcium dependent growth at 37°C (Higuchi and Smith 1961), presence of the virulence plasmid (Holmes and Quigley 1981), autoagglutination and virulence for mice (Laird and Cavanaugh 1980).

## Results

#### *Isolation of Y. enterocolitica and Y. pseudotuberculosis*

Table 1 shows the incidence of *Y. enterocolitica* and *Y. pseudotuberculosis* in the 406 rodents tested. Of the 83 positive isolates 59 were identified as *Y. enterocolitica* and 24 as *Y. pseudotuberculosis*

Table 2 shows the incidence of the above two *Yersinia* spp. in the 725 cat feces samples tested. Of the 64 isolates 19 were identified as *Y. enterocolitica* and 45 as *Y. pseudotuberculosis*.

The rate of positive isolates for the two *Yersinia* spp. from rodents was higher than that from cat feces. More *Y. pseudotuberculosis* than *Y. enterocolitica* isolates were obtained from cat feces, whereas more *Y. enterocolitica* isolates were found in rodents.

**Table 1.** Incidence of *Y. enterocolitica* and *Y. pseudotuberculosis* in rodents

Region	Rodent species	No. of rodents examined	No. of rodents positive	% positive	No. of rodents with	
					<i>Y. enterocolitica</i>	<i>Y. pseudotuberculosis</i>
Al-Ammariah	<i>R. rattus</i>	27	6	22.2	4	2
	<i>M. libycus</i>	13	4	30.8	3	1
Al-Dariyah	<i>R. rattus</i>	42	8	19	5	3
Al-Washim	<i>R. rattus</i>	29	7	14.1	5	2
Sultanah	<i>R. rattus</i>	65	7	10.8	6	1
Manfuha	<i>R. rattus</i>	73	21	28.8	13	8
Al-Rabwa	<i>R. rattus</i>	22	4	18.2	3	1
Al-Khaleeg	<i>G. gerbillus</i>	13	2	15.4	2	0
	<i>M. libycus</i>	29	4	13.8	2	2
Al-Nadheem	<i>G. gerbillus</i>	18	3	16.7	3	0
Al-Thomamah	<i>R. rattus</i>	43	9	20.9	6	3
	<i>G. gerbillus</i>	18	5	27.8	4	1
	<i>M. crassus</i>	14	3	21.4	3	0
Total		406	83	20.4	59	24

**Table 2.** Incidence of *Y. enterocolitica* and *Y. pseudotuberculosis* in cat feces

Region	No. of samples examined	No. of samples positive	% positive	No. of samples positive for:	
				<i>Y. enterocolitica</i>	<i>Y. pseudotuberculosis</i>
Al-Ammariah	47	5	10.6	2	3
Al-Dariyah	95	8	8.4	2	6
Al-Washim	95	9	9.5	3	6
Sultanah	100	9	9	3	6
Manfuha	92	13	14.1	5	8
Al-Rabwa	86	6	7	1	5
Al-Khaleeg	102	11	10.8	3	8
Al-Nadheem	55	4	7.3	2	2
Al-Thomamah	53	4	7.5	0	4
Total	725	64	8.8	19	45

#### Virulence associated determinants

All the *Yersinia* positive samples, *i.e.*, 23 rodents and 64 cat feces samples, were tested for virulence. Samples that gave a positive reaction did so for all the different virulence tests, *i.e.*, virulence plasmid, autoagglutination, calcium dependent growth

at 37°C and virulence for mice (Table 3). The rate of the virulent *Y. enterocolitica* and *Y. pseudotuberculosis* rodent isolates were found to be 10.2% (6 out of 59) and 79.2% (19 out of 24), respectively. Whereas, for cat feces isolates it was found to be 42.1% (8 out of 19) and 86.7% (39 out of 45), respectively. For both species, more virulent isolates were obtained from cat feces than from rodents and the number of virulent *Y. pseudotuberculosis* isolates were more numerous than those of *Y. enterocolitica*.

**Table 3.** Results of the virulence tests for *Y. enterocolitica* and *Y. pseudotuberculosis* isolates

<i>Yersinia</i> spp. and source	No. tested	No. positive for				
		Virulence plasmid	Autoagglutination at 37°C	Calcium dependent growth at 37°C	Virulence for mice	% virulent
<i>Y. enterocolitica</i>						
Rodents	59	6	6	6	6	10.2
Cat feces	19	8	8	8	8	42.1
<i>Y. pseudotuberculosis</i>						
Rodents	24	19	19	19	19	79.2
Cat feces	45	39	39	39	39	86.7

### Seasonal incidence

Table 4 shows the monthly incidence of *Y. enterocolitica* and *Y. pseudotuberculosis* in cat feces and *Rattus rattus* from the Manfuha region during 1991. The highest incidence of both species was in the cold months (December to February). *Y. enterocolitica* was isolated from 7 (4.67%) of the 150 cat feces samples and from 30 (20%) of the 150 rats. *Y. pseudotuberculosis* was isolated from 13 (8.67%) of the 150 cat feces samples and from 16 (10.67%) of the 150 rats. The lowest incidence of both species occurred in the summer (June to August), *Y. enterocolitica* was isolated from 1 (0.67%) of the 150 cat feces samples and from 18 (12%) of the 150 rats, *Y. pseudotuberculosis* was isolated from 6 (4%) of the 150 cat feces samples and from 8 (5.3%) of the 150 rats.

### Discussion

This report is the first evidence that *Y. enterocolitica* and *Y. pseudotuberculosis* are present in cat feces and in small rodents in Saudi Arabia.

**Table 4.** Monthly temperature and the incidence of *Y. enterocolitica* and *Y. pseudotuberculosis* in 150 specimens of *Rattus rattus* and 150 samples of cat feces

Month	Avg mo temp. (°C)	No. of cat feces positive for:		No. of rats positive for:	
		<i>Y. enterocolitica</i>	<i>Y. pseudotuberculosis</i>	<i>Y. enterocolitica</i>	<i>Y. pseudotuberculosis</i>
January	22.3	2	4	10	5
February	23.9	2	5	9	6
March	28.0	1	4	8	2
April	34.2	2	3	8	3
May	37.9	1	3	6	2
June	42.2	1	3	7	3
July	43.7	0	1	5	2
August	42.6	0	2	6	3
September	38.5	1	1	6	4
October	34.4	0	2	8	3
November	28.2	1	2	7	5
December	25.1	3	4	11	5

In the U.S.A., Shayegani *et al.* (1986), testing 1,426 animals including mice, recovered 148 isolates of *Y. enterocolitica* and related species. Bercovier *et al.* (1978) recovered 459 *Yersinia* strains from 507 small animals. Fukushima *et al.* (1990) recovered *Y. enterocolitica* and *Y. pseudotuberculosis* from mice in Japan at a rate of 53.9% and 4.6%, respectively. Kapperud (1975) recovered 24 isolates of *Y. enterocolitica* from 551 small rodents in Norway, Sweden, and Finland. Kaneko and Hashimoto (1981) recovered one isolate of *Y. pseudotuberculosis* and 15 isolates of *Y. enterocolitica* from a total of 16 isolate out of 495 small wild animals in Hokkaido, Japan. Reports of the isolation of *Y. enterocolitica* and *Y. pseudotuberculosis* from cats or cat stools are few and all came from Japan (Fukushima *et al.* 1989, Yanagawa *et al.* 1978).

Seasonal fluctuation of *Yersinia* spp. has been noted by several authors; most frequently maxima occur during the cold months in Japan (Fukushima *et al.* 1984, Linuma *et al.* 1992, Kaneko *et al.* 1978), the U.S.A. (Metchock *et al.* 1991), and Australia (Slee *et al.* 1992). The present study shows that also in Saudi Arabia the rate of isolation of *Y. enterocolitica* and *Y. pseudotuberculosis* from rodents or cat feces was highest in the winter. Thus, variations in temperature do influence the spread of the organisms among cats and rodents and these cats and rodents may act as carriers in the cold months. The Saudi Arabian winter temperatures are considered high as compared to those in Japan, the U.S.A. and Australia and, therefore, one would expect that the *Yersinia* spp. should be less prevalent in this country. The reasons behind the prevalence of *Yersinia* spp. during the cold season are not understood.

However, they could be related to an increase of host susceptibility or an increase of pathogenicity of the organism.

More *Y. enterocolitica* than *Y. pseudotuberculosis* isolates were obtained from rodents which is in agreement with the results reported by Fukushima *et al.* (1990). On the other hand, more *Y. pseudotuberculosis* than *Y. enterocolitica* isolates were isolated from cat feces, there are, however, no reports available for comparison. The virulence tests indicate that virulent isolates of *Y. pseudotuberculosis* from both rodents and cat feces are more numerous than virulent isolates of *Y. enterocolitica*.

The relationship between human infection with *Y. pseudotuberculosis* and *Y. enterocolitica* and the presence of these two organisms in domestic pets, farm animals and wild animals is not well understood. However, *Y. pseudotuberculosis* infection has been shown to occur through water contaminated by mice or cats (Fukushima *et al.* 1988, 1989).

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مدى وجود البكتيريا  
*Yersinia pseudotuberculosis* و *Yersinia enterocolitica*  
 في القوارض ومخلفات القطط بمنطقة الرياض -  
 المملكة العربية السعودية

علي عبدالله السلامة

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 ص.ب (٢٤٥٥) - الرياض ١١٤٥١ - المملكة العربية السعودية

سلالات البكتيريا *Yersinia enterocolitica* و *Y.pseudotuberculosis* مسببات رئيسية لالتهابات المعدة والأمعاء بما في ذلك اللفائفي الطرقي، كما انها تسبب التهاب كاذب للزائدة الدودية. ومع أن الاصابات الناجمة عن تلك البكتيريا قد ازدادت في كثير من دول العالم فإن التقارير التي توحى بعزلها من المملكة العربية السعودية ودول الخليج الأخرى تكاد تكون معدومة، لذلك فإن هذا المختبر قد سلط الدراسة عليها في سلسلة من الأبحاث. وبما أن الحيوانات وخصوصاً القوارض هي أحد المصادر الرئيسية لهذه البكتيريا إلى الانسان فلقد تمت هذه الدراسة لبيان مدى وجودها في ٤٠٦ من القوارض و٧٢٥ من مخلفات القطط المحصول عليها من منطقة الرياض، المملكة العربية السعودية، ووجدت تلك البكتيريا في ٨٣ (٨, ٢٠٪) من القوارض وفي ٦٤ (٨, ٨٪) من مخلفات القطط التي تم اختبارها. العزلات البكتيرية من القوارض شملت ٥٩ عزلة من البكتيريا *Y.entrocolitica* و٢٤ عزلة من البكتيريا *Y.pseudotuberculosis* بينما العزلات البكتيرية من مخلفات القطط شملت ١٩

عزلة من البكتيريا *Y.entrocolitica* و ٤٥ عزلة من البكتيريا *Y.pseudotuberculosis* . معدل العزلات الممرضة من البكتيريا *Y.entrocolitica* و *Y.pseudotuberculosis* التي حصل عليها من القوارض كان ٢, ١٠٪ و ٢, ٧٩٪ على التوالي، بينما كان معدل العزلات الممرضة التي حصل عليها من مخلفات القطط ١, ٤٢٪ و ٧, ٨٦٪ على التوالي.