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RODENTS AS RESERVIORS OF BABESIOSIS IN URBAN AREAS OF LAHORE

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ABSTRACT

Babesiosis was recorded in 2.3 percent of the rodents in the Lahore - the second most populated city of Pakistan and the capital of Punjab. The highest prevalence was recorded during the August at all the four structures sampled for rats and mice. It was 8.7 percent at Residential houses (RH) 10.7 percent at flour mills (FM) and fruit/vegetable shops (F/VS), and 6.7 percent at departmental stores and grocery shops (DS/GS). Inter-structure variation in the prevalence rate was statistically non-significant. Rodents captured from November to April had no infection. Sex-wise infection rate was statistically different in all the structures. Infection rate was higher in mice (2.4 percent) than rats (2.3 percent) but the difference was non-significant. Findings suggest that infected rodents may become a general health hazard and suitable measures required to eradicate them.

Key words: Babesia, house rat, house mouse, flourmills, Lahore, residential houses, zoonoses.

INTRODUCTION

Babesiosis is vectored to humans by ticks that are ecto-parasites of rodents (Levine, 1971; Telford *et al*., 1993). *Babesia microti*, a species of rodent origin, has been recognized as an agent of human babesiosis in the United States (Dammin *et al*., 1981). Symptoms of the disease appear between 1 to 4 weeks after a person is bitten by infected ticks. The patient suffers from a gradual onset of malaise, anorexia, fatigue, mild to moderate fever, sweats, and myalgia (Ruebush *et al*., 1977a, b). No case of human babesiosis has been reported from Southeast Asia. This situation may be attributed to the fact that neither any serological survey of the rodents nor of humans has been conducted in the region. The present study provides the first report on the prevalence of babesiosis in rodents from this region.

MATERIALS AND METHODS

Three localities of the Lahore metropolis were studied for the prevalence of babesial infection in rats and mice. These localities included (a) Allama Iqbal Town, (b) Walled City of Lahore, and (c) the areas of old city adjacent to the Lahore Railway Station.

Trapping of the rodents: Four types of structures viz., residential houses (RH), flour mills (FM), fruit /vegetable shops (F/VS), and the departmental stores/grocery shops (DS/GS) were sampled for rats and mice populations in each of these three localities. Twenty five live traps were set at each of these structures per night per month. The traps were baited with bread soaked in cooking oil, fresh vegetables, fruits and peanuts butter. The traps were set at dusk and collected at next dawn. A total of 3190 specimens of the house rats and 410 specimens of the house mouse were trapped; the trap success being 100%. Each live trap with captured animal was tagged indicating locality, structure and date of collection. Each specimen was physically restrained to determine its species and age group (Roberts, 1997).

Restraining and preparation of blood smears from rats and mice: Rodents were anaesthetized by putting the live traps in a thick transparent polythene bag that helped to observe the movement of animals. A cotton swab soaked in ether or chloroform as described by Singla *et al*. (2008) was used for anaesthetizing the animals. The body weight and measurements such as ear, tail, head and body length and hind foot length were taken. Species identification, sex and age determination was done as described by Roberts (1997).

For microscopic examination blood samples were collected from the ear tip puncture or cutting the tip of tail or foot pads of properly anesthesized rodent strictly following the aseptic measures (Edrissian *et al.,* 1982).Thick and thin blood smears were made and examined under a 12 mm diameter cover slip at X-400 magnification using microscope for identification of *Babesia* spp. by using Giemsa stain (Ijaz *et al*. 1998; Coles, 1986).

Statistical analysis. χ^2 test was applied to compare month-wise, season-wise, structure-wise, age-wise, sex-wise and species-wise difference in the prevalence of babesial infection. SPSS v 13 was used for this purpose.

RESULTS

A total of 3600 rats (n = 3190) and mice (n = 410) were scanned for *Babesial* infection and 2.3% (n = 84) were found to be positive (Table 1). The infection rate was almost similar in the rodents captured from FM (2.7%), RH (2.6%) and F/VS (2.6%) with the lowest in those captured from DS/GS (1.6%). The highest monthly prevalence was recorded during the August at all the four structures sampled for rats and mice. It was 8.7% at RH, 10.7% at FM and F/VS, and 6.7% at DS/GS (Table. 1). Inter-structure variation in the prevalence rate was statistically non-significant. No case of Babesial infection was recorded from November to April. The combined season-wise prevalence (Fig. 1) was the highest during summer (5.1%) that was followed by autumn (4.2%), and spring (0.1%). During spring, the infection was recorded only from RH (1.3%) while the highest infection rate during summer was recorded from FM (5.8%) and F/VS (5.8%). The infection rate for the remaining two structures was also highest during summer. It was 5.3% at RH and 3.1% at DS/GS (Fig. 1). The combined monthly ($\chi^2 = 39.10$; df = 11; $P < 0.01$) and seasonal ($\chi^2 = 28.21$; df = 3; $P < 0.01$) prevalence of the disease was statistically significant.

Sex-, age- and species-wise prevalence of *Babesial infection* in rats and mice trapped from four types of structures sampled for rats and mice is given in Fig. 1. Of the 3600 commensal rodents, 2.1 percent of the females (n = 43) and 2.7 percent of the males (n = 41) were infected. Sex-wise infection rate was significantly different in all structures. The infection rate for females was 2.2, 2.3, 2.6 and 1.2 percent at RH, FM, F/VS and DS/GS whereas in case of males, it was 3.0, 3.2, 2.4 and 2.0 percent at RH, at FM, at F/VS at GS/DS, respectively. None of the young was infected while 2.6 percent of the adults caught from all the four structures were infected. The highest infection rate in adults was recorded from FM (3.0%) which was followed by 2.9 percent in F/VS, 2.8 percent in RH and 1.8 percent in DS/GS. The infection rate in mice was higher (2.4%) than rats (2.3%) but the difference was non-significant. The infection rate in mice was highest in those from F/VS (3.0%), followed by RH (2.5%), FM (2.2%) and DS/GS (2.1%), where as the house rats inhabiting DS/GS were the least infected (1.5%).

Locality-related variations in the prevalence of *Babesial infection*

Allama Iqbal Town; A total of 1200 rodents were examined for the presence of *Babesia* infection by identification of adults and various stages of *Babesia* spp. in red blood cell. Of these, seventeen (1.4%) were found positive for babesial infection (Table 1). Inter-structure infection rate varied significantly. The prevalence was recorded during summer (2.7%) and autumn (3.0). The lowest infection rate (1.3%) of was recorded during at DS/GS of Allama Iqbal Town while the highest (4.0%) was recorded during at FM. 1.2 percent of the females (n = 8) and 1.7 percent of the males (n = 9) were infected. No young was infected however, 1.6 percent of adult rats and mice were infected. The infection rate was 1.5, 2.4, 1.9 and 0.7 percent in adults caught from RH, FM, F/VS and GS/DS, respectively. The infection rate was almost similar in rats (1.4%) and mice (1.5%).

Walled City: The highest prevalence rate (3.3%) was observed at F/VS followed by RH and FM (2.6%), and the lowest at DS/GS (1.7%). The infection rate was not different significantly. The highest (10.0%) month-wise prevalence was noted during August and September whereas the lowest (1.0%) was recorded during June. No infection was recorded from November through May (Table 1). Statistically significant difference was noted was month and season-wise infection rate was compared. 2.2 percent of the females (n = 15) and 3.0 percent of the males (n = 16) were infected (Fig. 1). None of the young was infected whereas 2.9% of the adults were infected. It is also evident that prevalence was lower in mice (1.7%) than in rats (2.7%), the difference was however statistically non-significant.

Railway Station / Adjacent Areas: The highest prevalence (3.7%) was recorded at RH that was followed by FM (3.3%), F/VS (2.7%) and DS/GS (2.3%). The difference in inter-structure infection rate was non-significant. The highest prevalence was noted during autumn (3.0%) and the lowest during spring (0.3%) whereas no positive case was detected during winter (Fig. 1). 2.9 percent of the females (n = 20) and 3.2 percent of the males (n = 16) were infected. None of the young was infected whereas 3.4 percent of the adults were infected (Fig. 1). The prevalence was higher in mice (4.9%) than rats (2.8%).

DISCUSSION

Blood parasites are common in domestic animals, rodents and human beings, may be fatal and mostly transmitted by ectoparasites to human and other (Bossi *et al*., 2002; Rios *et al.,* 2003; Barreira *et al.,* 2004). Babesia species are transmitted by ticks to susceptible animals, rodents and humans (Homer *et al*., 2000; Karbowiak, 2004). Although a number of different animals serve as reservoirs of Babesia species however rodents are at the top (Karbowiak, 2004).

The disease has been studied very extensively in Europe, United States (Anderson *et al*., 1974; Benach *et al*., 1978; Fitzpatrick *et al*., 1968, 1969; Garnham, 1980; Meldrum *et al*., 1982; Scharfman *et al*., 1977; Scholtens *et al*., 1968; Skrabalo and Deanovic, 1957; Steketee *et al*., 1985) and elsewhere in the world (Wei *et al.*, 2001; Yokoyama *et al*., 2003). Present research was planned to

have an assessment about the risk involeved in transfer of Babesia species to humans living in close association with rodents at three localities of Lahore, Pakistan. Overall prevalence of *Babesia* infection in captured rodents was 2.3% per cent. There were not significant differences in prevalence peaks of Babesia infection of rats/mice trapped from four structures of Allama Iqbal town (1.4%), Walled City (2.6%) and Railway Station/adjacent area (3.0%). In contrast higher infection rates had been observed by different research workers working in various geographical regions. Babesial infection in rodents observed by Shih *et al.* (1997) was 83 per cent in captured rodents at Taiwan. Sinski *et al.* (2006) reported marked differences in prevalence of babesiosis in three rodents ranging from 1 to 42 per cent. The prevalence rate of 15.9% and 11.8% was recorded in two different rodents by Duh *et al*. (2005) in Europe. Similarly it was 27.3% in the findings of Gazeta *et al.* (2004) and 15.5% Maml *et al.* (2007) at Southeastern Brazil. Many variations are there in the prevalence of Babesiosis in rats/mice of different geographical areas suggesting that it is not dependent on the locality rather the presence or absence of vector responsible for the transmission of parasite.

The highest (9.0%) month-wise prevalence was noted during August while no infection was recorded during January, February, March, April, November and December. The highest (5.0%) seasonal prevalence was observed during summer followed by autumn (4.2%) while the lowest in spring no infected rodent was trapped in winter. The results of the present study are in conformity with Gubler *et al.* (2001), Fichet-Calvet *et al.* (2003), Karbowaik (2004) and Bajer *et al.* (2005). This variation of seasonal peaks is in accord with the presence of ticks and nil, if ticks are absent. The transfer as well as maintenance of infection in rodents depends on the contaiminated environment and presence of ticks in the area. Occurrence of blood protozoan documented by Morsy *et al.* (1994) in *R. rattus* was (34.5%) and 9-33% in *Microtus agrestis* by Karbowiak (2004). It is evident from literature and our findings that presence and rise of Babesial ifection in rodents is not correlated with the season and presence of ticks is obligatory. Most important reservoirs of Babesia infection are rodents as was reported by Borggraefe *et al*. (2006). The variation in prevalence of parasite in rodents and ticks is changeable and influenced by season in which number of ticks are more.

Similarly, minor differences were in the prevalence of parasite in males (2.7%) and female rats/mice (2.0%). As regard age-wise prevalence, it was present only in adults comparable with the findings of Gubler *et al.* (2001) and Karbowaik (2004). Relation of

Table 1. Combined monthly variations (v) in the prevalence of Babesia spp. recorded in rats and mice captured from (i) residential houses, (ii) flour mills, (iii) fruit/vegetable shops and (iv) departmental stores of (a) Allamma Iqbal Town, (w) walled city of Lahore (r) Lahore railway station and their combined prevalence at each locality (v) and (c) locality in the Lahore.

Structure/ Locality	Month												Overall
	D	J	F	M	A	M	J	J	A	S	O	N	
i(a)	-	-	-	-	-	-	-	4.0	4.0	4.0	4.0	-	1.3
ii(a)	-	-	-	-	-	-	-	4.0	8.0	8.0	4.0	-	2.0
iii(a)	-	-	-	-	-	-	-	-	8.0	8.0	4.0	-	1.7
iv(a)	-	-	-	-	-	-	-	-	4.0	4.0	-	-	0.7
v(a)	-	-	-	-	-	-	-	2.0	6.0	6.0	3.0	-	1.4
i(w)	-	-	-	-	-	-	-	8.0	8.0	12.0	4.0	-	2.7
ii(w)	-	-	-	-	-	-	-	4.0	12.0	12.0	4.0	-	2.7
iii(w)	-	-	-	-	-	-	4.0	12.0	12.0	12.0	-	-	3.3
iv(w)	-	-	-	-	-	-	-	4.0	8.0	4.0	4.0	-	1.7
v(w)	-	-	-	-	-	-	1.0	7.0	10.0	10.0	3.0	-	2.6
i (r)	-	-	-	-	-	4.0	4.0	8.0	12.0	8.0	8.0	-	3.7
ii (r)	-	-	-	-	-	-	-	12.0	12.0	8.0	8.0	-	3.3
iii (r)	-	-	-	-	-	-	-	4.0	12.0	8.0	8.0	-	2.7
iv (r)	-	-	-	-	-	-	-	4.0	8.0	8.0	8.0	-	2.3
v (r)	-	-	-	-	-	1.0	1.0	7.0	11.0	8.0	8.0	-	3.0
i. (c)	-	-	-	-	-	1.3	1.3	6.7	8.0	8.0	5.3	-	2.6
ii(c)	-	-	-	-	-	-	-	6.7	10.7	9.3	5.3	-	2.7
iii (c)	-	-	-	-	-	-	1.3	5.3	10.7	9.3	4.0	-	2.6
iv (c)	-	-	-	-	-	-	-	2.7	6.7	5.3	4.0	-	1.6
v (c)	-	-	-	-	-	0.3	0.7	5.3	9.0	8.0	4.7	-	2.3

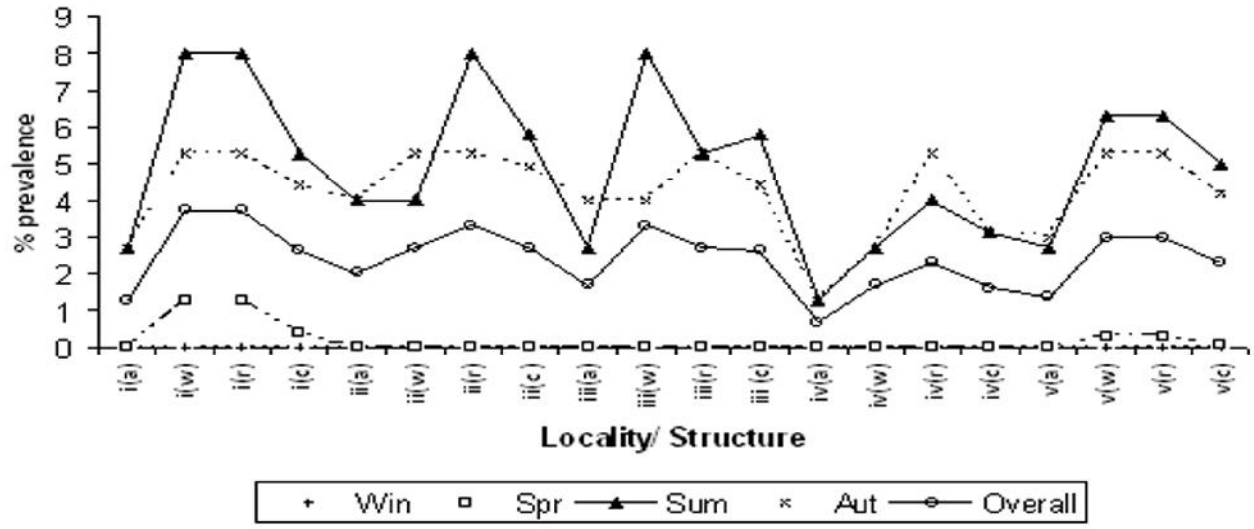

Fig. 1. Seasonal variations in the prevalence of rodent babesiosis in (i) residential houses, (ii) flour mills, (iii) fruit/vegetable shops and (iv) departmental stores of (a) Allamma Iqbal Town, (w) walled city of Lahore (r) Lahore railway station and their combined prevalence at each locality (v) and (c) locality in the Lahore.

sex and age of rodents with the prevalence rate of Babesial species is negligible rather presence of vector (tick) is mandatory for transfer of parasite from one host to the other. Prevalence peaks are higher in those areas where ticks were abundant in the environment of the rodents.

It was also noted that rats were susceptible to disease than mice as was also reported by El-Kady (1998) and Borggrafe *et al.* (2006). Minor differences may be due to variation in environmental conditions of the areas studied. Rats are abundant in the field where chances of contact with infected ticks are higher than mice. However the difference in infection rate was negligible.

On the basis of literature survey and findings of present work plan it can be concluded that rats/mice are important reservoir of infection and may play role in transmission on Babesiosis to humans.

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