Patho-physiological observations in natural concurrent infections of helminth parasites of zoonotic importance in the wild rodents, *Bandicota bengalensis*

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Abstract Screening of 83 mature wild rodents of *Bandicota bengalensis* (52 male and 31 females), live trapped from premises near railway station, fish market and agricultural fields in Ludhiana district of Punjab province, India was carried out to assess the natural occurrence of helminth parasites and their potential as reservoirs of zoonoses. On necropsy, intestine of rats was found infected with adult cestode parasites of *Hymenolepis diminuta* and *H. nana*. Liver was found infected with *Capillaria hepatica* and *Cysticercus fasciolaris*. These parasites were present either alone or as concurrent infection. In overall, 68.67 % rats were found infected of which 59.65 % were infected with nematode parasite, *C. hepatica* and 75.44 % with cestode parasites. Grossly, pathognomonic lesions of *C. hepatica* and *C. fasciolaris* were seen clearly and microscopic changes were observed only in severely infected cases. Overall investigation on the effect of these parasitic infections on pathophysiology of the host revealed that rodents serve as reservoirs of these parasitic infections without having much pathogenic effect on their vital organs, fecundity and enzyme activities. Because of its close association with human habitations, *B. bengalensis* may act as an important source of zoonotic infections and

thus direct or indirect contact with excrements and carcasses of this rodent species, should be avoided.

Keywords *Bandicota bengalensis* · Concurrence · Enzymatic levels · Helminth infection · Vital organs

Introduction

Rodents belonging to the largest and most successful group of mammals worldwide are cosmopolitan in distribution and have a high rate of reproduction. They significantly affect crop production and livelihoods of farmers in both developed and developing countries (Stenseth et al. 2003; Singla and Parshad 2010; Singla and Babbar 2010) in addition to playing a significant role in public health, chiefly act as carriers or reservoirs of microbes and parasites of zoonotic importance (Castillo et al. 2003; Singla et al. 2003, 2008a; Meerburg 2009). Increased rodent population in an area can be directly related to the increased zoonotic diseases in human population (Bradshaw 1999). The lesser bandicoot rat, *Bandicota bengalensis* is the predominant rodent pest species found throughout South East Asia in both agricultural and commensal situations. Individual rats are mostly found infected with more than one species of parasites (Singla et al. 2008b, 2013). The eggs of these parasites usually pass out in rodent feces in fields, grain stores and amongst food-stuffs in houses, and are responsible for disease spread (Khatoon et al. 2004). Thus, infection in humans generally occurs directly through contact with rodent excrement and through ingesting food and water contaminated with their feces and carcasses. But no authenticated investigation related to the effect of parasitic infections on pathology of vital organs, fecundity (reproductive parameters) and

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enzymatic activities is available for *B. bengalensis*. Thus the present study was planned to know the prevalence of natural helminth parasitic infections of zoonotic importance in *B. bengalensis* and their effect on pathophysiology of host.

Materials and methods

Collection and examination of rats

A total of 83 mature *B. bengalensis* (52 male and 31 female), live trapped from premises near railway station, fish market and agricultural fields at Ludhiana, Punjab province of India in the year 2013–2014 were maintained individually in laboratory cages of size 23" × 13" × 13" with ad libitum food and water. Food consisted of cracked wheat smeared with powdered sugar and groundnut oil in ratio 96:2:2. The rats were maintained as per the guidelines of Institutional Animal Ethics Committee for about 2-months.

All rats were apparently healthy and active. After 2 months of rearing, all the rats were humanely sacrificed and necropsied. Gross lesions were observed and recorded. Microscopic examination of cystic area of liver tissue was performed by tissue press technique for identifying the infection. A small portion of liver and intestine of infected rats were excised, fixed in neutral buffered formalin and processed into paraffin wax (Humason 1979). Sections (6μ) were routinely cut, stained with hematoxylin and eosin (H&E) and studied under light microscope. The fecal pellets of all the rats were examined by flotation and sedimentation techniques for the presence of eggs of parasites as described in Soulsby (1982).

The reproductive organs (testes, epididymis, seminal vesicles, prostate gland, ovaries and uterus) and vital organs (liver, spleen, kidneys and adrenal glands) of all the infected and uninfected rats were dissected out, cleared of fat and blotted dry to determine their weights (g/100 g bw). Blood samples of all the rats were collected by cardiac puncture, centrifuged at 3000g for 10 min and serum collected was stored at −20 °C until analysis. Acid phosphatase (ACP), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (IU/L) were assayed as per the standard methods. One of the cauda epididymis of each of the infected and uninfected male rats was incised and pressed to take out the cauda epididymal fluid. The sperm count (millions/ml), motility (%), sperm viability (%) and sperm abnormality (%) were determined as per the methods described by Singla and Garg (2013).

Before necropsy, vaginal smear of all the female rats was examined daily for a period of 30 days to determine

the number of estrous cycles, duration (days) of one estrous cycle and the duration of different stages of the estrous cycle. The duration between two metestrous stages was considered as the duration of one estrous cycle, and the number of metestrous periods experienced was taken as a measure of the number of cycles completed.

Data analysis

Different values were determined as mean ± SD. Percent prevalence of different helminth infections was calculated. Significance of difference in patho-physiological effects of helminth infections between groups of rats having different kind and level of infection was determined using analysis of variance.

Results

A total 83 (52 males and 31 females) rats were examined and 68.67 % were found infected with four different helminth parasites alone and in concurrence with each other (Table 1). Female rats were found to be more infected (74.19 %) as compared to males (65.38 %). During necropsy investigations, gross lesions comprising of irregular pale cystic areas or streaks were found randomly scattered on the liver surface of 19 male (55.88 %) and 15 female (65.22 %) rats. On further investigation, it became apparent that these rats were infected with *C. hepatica*. Out of these infected rats, 9 male and 8 female rats were having concurrent infection of *Cysticercus fasciolaris* (a metacestode of *Taenia taeniaeformis*), *Hymenolepis diminuta* and *H. nana* whereas, 2 males were having concurrent infection of *H. diminuta* and *H. nana* and one female rat was having concurrent infection of *H. diminuta*. Infection of *C. fasciolaris* was evident in the form of whitish cysts on the liver. *C. fasciolaris* infection was detected on opening the cysts, when the larvae burst out. Larvae were opaque white in colour and immature. Each cyst contained a single live characteristic strobilocercus larva. Figure 1 shows concurrent infection of *C. fasciolaris* and *C. hepatica* in the liver of one of the infected rat. Out of the total infected rats, eleven male (32.35 %) and eight female (34.78 %) rats were found infected with *C. fasciolaris* alone. Whereas, only three males (8.82 %) were infected with *H. diminuta* alone. *H. diminuta* and *H. nana* infections were found in the intestine. Fecal examination of rats revealed the presence of nematode parasite, *C. hepatica* eggs in 59.65 % (34) and cestode parasites of three species in 75.44 % (43) infected rats (Table 1).

During present investigation, no significant effect of parasitic infections was found on weights of reproductive and vital organs of male rats (Table 2). Two male rats

Table 1 Prevalence of different parasitic infections found in *B. bengalensis* of both sexes

Sex	No. infected/total	Percent infected	Parasites found	Animals infected	Percentage among infected individuals	Percentage among total individuals
Male rats	34/52	65.38	<i>Capillaria hepatica</i>	8	23.53	15.38
			<i>Cysticercus fasciolaris</i>	11	32.35	21.15
			<i>Hymenolepis diminuta</i>	3	8.82	5.77
			Concurrent infection ^a	9	26.47	17.31
			Concurrent infection ^b	2	5.88	3.85
			Concurrent infection ^c	1	2.94	1.92
			Nematode infection	19	55.88	36.54
			Cestode infection	26	76.47	50.00
Female rats	23/31	74.19	<i>Capillaria hepatica</i>	6	26.09	19.35
			<i>Cysticercus fasciolaris</i>	8	34.78	25.81
			Concurrent infection ^a	8	34.78	25.81
			Concurrent infection ^d	1	4.35	3.23
			Nematode infection	15	65.22	48.39
			Cestode infection	17	73.91	54.84
			<i>Capillaria hepatica</i>	14	24.56	16.87
			<i>Cysticercus fasciolaris</i>	19	33.33	22.89
Total	57/83	68.67	<i>Hymenolepis diminuta</i>	3	5.26	3.61
			Concurrent infection ^a	17	29.82	20.48
			Concurrent infection ^b	2	3.51	2.41
			Concurrent infection ^c	1	1.75	1.20
			Concurrent infection ^d	1	1.75	1.20
			Nematode infection	34	59.65	40.96
			Cestode infection	43	75.44	51.81

^a Concurrent infection of *Cysticercus fasciolaris*, *Capillaria hepatica*, *Hymenolepis diminuta* and *H. nana*

^b Concurrent infection with *Capillaria hepatica*, *Hymenolepis diminuta* and *Hymenolepis nana*

^c Concurrent infection with *Cysticercus fasciolaris* and *Hymenolepis diminuta*

^d Concurrent infection with *Capillaria hepatica* and *Hymenolepis diminuta*

having concurrent infection of *C. hepatica*, *H. diminuta* and *H. nana* were, however, found to have enlarged spleen. No significant effect of parasitic infections was observed on sperm parameters such as sperm motility, viability, density and morphology as well enzyme levels of ALP, AST and ALT. The level of ACP was, however, found to be significantly high in rats having concurrent infection of all the four parasites and those infected only with *H. diminuta*.

One of the female rat was found to be heavily infested with *C. hepatica* as revealed by severity of pale cystic areas on the liver (Fig. 2). It was also having concurrent infection of *H. diminuta* and enlarged spleen and kidney. No significant effect of parasitic infection was observed on weights of other visceral organs. During present investigation, no significant effect of these parasitic infections was observed on duration of estrous cycle at different stages and levels of ACP, ALP, AST and ALT between infected and uninfected female rats (Table 3).

Fig. 1 Liver showing concurrent infection of *Capillaria hepatica* (arrow heads) and *Cysticercus fasciolaris* (arrow)

Table 2 Effect of parasitic infections on weights of different organs, sperm parameters and enzymatic activities in male rats

Parameters	Uninfected (n = 18)	<i>Cysticercus fasciolaris</i> (n = 11)	<i>Capillaria hepatica</i> (n = 8)	<i>Hymenolepis diminuta</i> (n = 3)	Concurrent infection (n = 9) ^a	Concurrent infection (n = 2) ^b	Concurrent infection (n = 1) ^c
Organ weights (g/100 g bw)							
Testis	0.22 ± 0.03	0.17 ± 0.02	0.21 ± 0.02	0.13 ± 0.06	0.16 ± 0.02	0.19 ± 0.08	0.22 ± 0.00
Epididymis	0.06 ± 0.00	0.06 ± 0.01	0.07 ± 0.002	0.05 ± 0.01	0.05 ± 0.003	0.06 ± 0.01	0.04 ± 0.00
Seminal vesicles	0.47 ± 0.03	0.40 ± 0.05	0.50 ± 0.06	0.24 ± 0.06	0.42 ± 0.06	0.44 ± 0.09	0.06 ± 0.00
Prostate gland	0.26 ± 0.03	0.20 ± 0.04	0.30 ± 0.04	0.15 ± 0.07	0.20 ± 0.03	0.18 ± 0.09	0.05 ± 0.00
Liver	3.89 ± 0.12	4.14 ± 0.17	4.31 ± 0.20	4.07 ± 0.13	3.95 ± 0.19	3.34 ± 0.38	3.48 ± 0.00
Spleen	0.33 ± 0.02	0.35 ± 0.04	0.32 ± 0.02	0.43 ± 0.02	0.39 ± 0.02	0.64 ± 0.15*	0.33 ± 0.00
Kidney	0.32 ± 0.01	0.32 ± 0.02	0.33 ± 0.02	0.35 ± 0.02	0.28 ± 0.02	0.32 ± 0.05	0.44 ± 0.00
Adrenal gland	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.002	0.01 ± 0.000	0.01 ± 0.001	0.01 ± 0.002	0.03 ± 0.00
Sperm parameters							
Sperm motility (%)	39.41 ± 7.40	17.27 ± 5.64	33.13 ± 8.05	4.00 ± 2.09	25.89 ± 9.09	36.75 ± 20.68	6.50 ± 0.00
Sperm viability (%)	48.11 ± 6.46	29.40 ± 3.49	44.48 ± 6.19	16.48 ± 2.54	36.20 ± 7.26	51.96 ± 16.37	23.30 ± 0.00
Sperm density (×10 ⁶ /ml)	277.65 ± 24.51	210.45 ± 16.81	271.25 ± 27.17	176.67 ± 26.28	245.00 ± 20.68	212.50 ± 19.45	180.00 ± 0.00
Sperm abnormality (%)	47.33 ± 7.06	52.18 ± 6.40	35.46 ± 5.83	80.17 ± 4.29	58.86 ± 10.10	52.45 ± 24.46	52.88 ± 0.00
Enzymatic levels (IU/L)							
ACP	48.05 ± 6.99	41.18 ± 4.78	31.06 ± 2.58	82.24 ± 6.65*	67.93 ± 8.96*	44.93 ± 17.45	36.01 ± 0.00
ALP	71.75 ± 5.63	66.45 ± 6.40	72.83 ± 4.45	74.48 ± 8.55	80.38 ± 13.30	83.90 ± 11.42	58.33 ± 0.00
ALT	27.42 ± 2.59	24.22 ± 3.23	23.72 ± 3.32	29.60 ± 3.18	27.92 ± 1.91	24.78 ± 6.03	22.99 ± 0.00
AST	36.56 ± 1.87	38.96 ± 2.62	42.23 ± 1.97	44.60 ± 2.04	36.31 ± 2.37	34.63 ± 4.45	31.48 ± 0.00

Values are mean ± SE; n = number of rats

ACP, acid phosphatase, ALP alkaline phosphatase, AST aspartate aminotransferase, ALT alanine aminotransferase

* Significant difference from uninfected group of rats (p < 0.05)

^a Concurrent infection with *Cysticercus fasciolaris*, *Capillaria hepatica*, *Hymenolepis diminuta* and *Hymenolepis nana*

^b Concurrent infection with *Capillaria hepatica*, *Hymenolepis diminuta* and *Hymenolepis nana*

^c Concurrent infection with *Cysticercus fasciolaris* and *Hymenolepis diminuta*

Eggs of *H. nana* and *H. diminuta* as found in the feces of male and female rats have been shown in Figs. 3a and b, respectively. Figure 3c shows the scolex of *H. nana* containing hooks and suckers used for attachment to the intestinal wall and Fig. 3d shows the section of intestine of the rat having infections of *H. nana*. Figure 3e shows a rat having concurrent infection of *C. fasciolaris* and *H. diminuta* in the liver and intestine, respectively. In the present study, massive deposition of characteristic *C. hepatica* eggs having bipolar caps was found in the parenchyma of the liver of infected rats. Microscopically, the infected liver revealed granulomatous reaction around eggs giving rise to several microgranulomas or coalescing macrogranulomas. The microgranulomas consisted of aggregates of eggs surrounded by fibrous connective tissue with few lymphomononuclear cells. The adjacent parenchyma of the affected liver showed granular degeneration (Figs. 3f). Whereas, mild degeneration was observed

in the parenchyma of liver infected with *C. fasciolaris* (Fig. 3g). The section of intestine of rat infected with *H. nana* showed degeneration and goblet cell hyperplasia of intestine (Fig. 3h).

Discussion

During the present necropsy investigations, gross lesions comprising of irregular pale cystic areas or streaks were found randomly scattered on the liver surface of rats. On further investigation, these rats were apparently found infected with *C. hepatica*. The eggs of *C. hepatica* accumulate in liver in the form of irregular white or yellow patches and streaks on the external surface (Gotardo et al. 2000).

Host gender also has significant effect on parasite infection patterns. Present investigation reveals higher percent infection in female rats compared to male rats. On

the contrary, Wilson et al. (2002) and Skorpung and Jensen (2004) have reported higher rates of parasitism in males than females. Sex biases in parasitism rates may be caused by physiological differences between males and females, such as in the levels of a number of steroid hormones

Fig. 2 Liver showing severe infection of *Capillaria hepatica* in the form of pale cystic areas

which were known to have direct or indirect effects on components of the immune system and/or on parasite growth and development. Other mechanisms generating sex bias include differences in body size, body composition and behaviour (Rosa et al. 2007).

The present study showed concurrent infection of *C. hepatica* and *H. diminuta* with enlarged spleen and kidneys. Persistent fever, hepatomegaly, leucocytosis with eosinophilia, anemia, pulmonary symptoms, splenomegaly, enlargement of the kidneys and wasting have also been reported in hepatic capillariasis (Juncker-Voss et al. 2000). Studies on laboratory mice have indicated that the *C. hepatica* may increase mortality and decrease fecundity (Singleton and Spratt 1986). However, during the present investigation no significant effect of *C. hepatica* infection on reproductive parameters of male and female rats was found. The fertility, mortality and behaviour of the host population depend on how the parasites were distributed within it (Hudson and Dobson 1995). The first human case of hepatic capillariasis, was reported in Korea (Choe et al. 1993). All together, only 37 cases of human capillariasis

Table 3 Effect of parasitic infections on weights of different organs, estrous cycle and enzymatic activities in female rats

Parameters	Uninfected (n = 8)	<i>Cysticercus fasciolaris</i> (n = 8)	<i>Capillaria hepatica</i> (n = 6)	Concurrent infection (n = 8) ^a	Concurrent infection (n = 1) ^b
Organ weights (g/100 g bw)					
Uterus	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.00
Ovary	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
Liver	4.55 ± 0.19	4.16 ± 0.19	3.83 ± 0.21	4.35 ± 0.37	4.83 ± 0.00
Spleen	0.37 ± 0.04	0.40 ± 0.03	0.44 ± 0.03	0.33 ± 0.04	1.14 ± 0.00*
Kidney	0.35 ± 0.03	0.37 ± 0.02	0.33 ± 0.02	0.36 ± 0.02	0.56 ± 0.00*
Adrenal gland	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Estrous cyclicity in 30 days duration					
No. of estrous cycles	5.88 ± 0.45	5.50 ± 0.66	6.00 ± 0.49	5.19 ± 0.48	6.00 ± 0.00
Duration of one estrous cycle (days)	5.56 ± 0.37	6.08 ± 0.58	5.50 ± 0.40	6.44 ± 0.43	5.00 ± 0.00
Proestrous stage (days)	5.69 ± 0.48	5.08 ± 0.65	5.80 ± 0.52	5.19 ± 0.34	6.00 ± 0.00
Estrous stage (days)	3.75 ± 0.56	3.08 ± 0.51	3.60 ± 0.67	3.13 ± 0.49	4.50 ± 0.00
Metestrous stage (days)	9.44 ± 0.54	9.67 ± 0.55	9.40 ± 0.65	9.44 ± 0.24	8.50 ± 0.00
Diestrous stage (days)	11.63 ± 0.59	12.67 ± 0.78	11.60 ± 0.71	12.75 ± 0.52	11.00 ± 0.00
Enzymatic levels (IU/L)					
ACP	82.94 ± 3.43	80.95 ± 3.48	82.58 ± 3.97	86.05 ± 2.78	87.35 ± 0.00
ALP	296.91 ± 7.81	311.45 ± 11.43	324.21 ± 17.89	321.25 ± 11.58	288.49 ± 0.00
ALT	58.04 ± 3.80	62.83 ± 8.42	53.67 ± 6.72	61.38 ± 5.32	60.60 ± 0.00
AST	33.00 ± 3.84	41.23 ± 5.50	32.11 ± 5.88	37.61 ± 4.58	26.17 ± 0.00

Values are mean ± SE; n = number of rats

ACP acid phosphatase, ALP alkaline phosphatase, AST aspartate aminotransferase, ALT alanine aminotransferase

* Significant difference from uninfected group of rats (p < 0.01)

^a Concurrent infection with *Cysticercus fasciolaris*, *Capillaria hepatica*, *Hymenolepis diminuta* and *Hymenolepis nana*

^b Concurrent infection with *Capillaria hepatica* and *Hymenolepis diminuta*

◀ **Fig. 3** **a** Characteristic eggs of *Hymenolepis nana*; **b** characteristic eggs of *H. diminuta*; **c** typical scolex of *H. nana*; **d** rat showing cyst of *Cysticercus fasciolaris* in liver and *H. diminuta* infection in intestine; **e** section of intestine showing *H. nana* infection, H & E. 4×; **f** section of liver with eggs of *Capillaria hepatica* showing microgranulomas surrounded by fibrous tissue, H & E. 10×; **g** section of liver showing *Cysticercus fasciolaris* infection and mild degeneration in the adjacent liver parenchyma, H & E. 10×; **h** section of intestine infected with *H. nana* showing degeneration and goblet cell hyperplasia, H & E. 10×

have been reported worldwide. The disease, however, usually remains misdiagnosed or undetected (Slais 1974).

Cysticercus fasciolaris is found in the liver of rodents in the form of cysts (Soulsby 1982). Its infection is clinically asymptomatic and is considered harmless (Singla et al. 2003). In the present study only mild degenerative changes were observed in liver infected with *C. fasciolaris*. *H. diminuta*, is the tapeworm, whose definitive hosts are rodents. Eggs of *H. diminuta* can be dispersed in the environment via beetle feces (Pappas and Barley 1999), thereby representing an additional source of infections and egg dispersal. Hymenolepiasis due to *H. diminuta* has been reported to affect about 36 million people worldwide (Peters and Pasvol 2002).

Rodents are the reservoir hosts of *H. nana*. Because of the unusual life cycle of this species, where no intermediate host is essential, man is probably at the main risk of infection. Although young children can be infected with *H. nana* eggs from rodent sources, this type of infection is probably less common (Faust et al. 1962). In the present study, *H. nana* has been found to cause degeneration of intestine.

During present investigation, high level of infection in male and female *B. bengalensis* was found with helminth parasites alone and in concurrence with each other. Three helminth species found during present study i.e. *H. diminuta*, *H. nana* and *C. hepatica* have been reported as zoonotic (Stojcevic et al. 2004; Waugh et al. 2006; Easterbrook et al. 2007; Hancke et al. 2011) thus representing a risk to public health. Overall investigation on the effect of these parasitic infections on pathophysiology of the host revealed that rodents may serve as reservoirs of these parasitic infections without having much pathogenic effect on their vital organs, fecundity and enzyme activities. Concurrent infection of *C. hepatica*, *C. fasciolaris*, *H. diminuta* and *H. nana* along with patho-physiological observations in their host is the first report of its kind. Because of its close association with human habitations, *B. bengalensis* may act as an important source of zoonotic infections and thus due care should be taken to avoid direct or indirect contact with excrements and carcasses of this pest species.

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