RESEARCH ARTICLE

Concurrent infection of *Cryptosporidium* and *Giardia* in synanthropic rodents: First report from Punjab, India

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Abstract

The present morphometric coproscopic study was aimed to investigate the protozoan infections in synanthropic rodents of urban areas from Punjab. Faecal samples of total 65 rodents of two species i.e. the house rat, *Rattus rattus* (n=40) and the lesser bandicoot rat, *Bandicota bengalensis* (n=25) collected from residences/shops, poultry farms and fish market at Ludhiana (Punjab), India were examined by formaldehyde–ether sedimentation and modified kinyoun acid fast stain for the presence of protozoan parasites. *Cryptosporidium* oocysts were detected in 32.30% rats comprising 36% *B. bengalensis* and 30% *R. rattus*. Morphometric examination of the acid-fast stained faecal smear revealed ovoid oocysts (average size of 7.70-9.8 x 5.5-7.0 µm) of *Cryptosporidium* whose morphology resembled that of *C. muris*. Precysts/cysts of *Giardia* sp. were also identified in 4.61% of the stained faecal smears. This is the first report of concurrent *C. muris* and *Giardia* sp. infections in synanthropic rodents of urban domestic and peridomestic area of Punjab, India.

Keywords: Coproscopic, *Cryptosporidium*, *Giardia,* Protozoans, Synanthropic rodents.

Ind J Vet Sci and Biotech (2021): 10.21887/ijvsbt.17.1.11

Introduction

Cryptosporidium and *Giardia* are two genera of parasitic protozoa capable of infecting humans and a wide variety of animal species, including pets and wildlife (Brar *et al*., 2017). Wildlife has received the least attention of these possible sources of pathogens and the risk posed by these populations to public health is not well understood. Rodents are considered as a major risk factor for public health by serving as a source of pathogens in the environment via leading to contamination of food, water and soil (Singla *et al*., 2008; Perec-Matysiak *et al*. 2015).

Cryptosporidium is an intracellular extra-cytoplasmic apicomplexan gastrointestinal coccidian pathogen of humans and animals having *C. parvum*, *C. hominis, C. muris* as important zoonotic species. *Giardia* responsible for gastrointestinal giardiosis exists in two forms i.e. actively multiplying pathogenic trophozoite form and infective cyst form (Sursal and Yildiz, 2020). Certain species of *Giardia* (*Giardia muris*, *G. duodenalis* and *G. microti*) harboured by rodents are vulnerable to humans and a wide variety of mammalian species (Helmy *et al.,* 2018). Worldwide, several studies have detected individual infections of *Cryptosporidium* and *Giardia* in rodents (Lv *et al.,* 2009; Perec-Matysiak *et al.,* 2015).

Epidemiological studies concerning these pathogens in wild rodents, apart from the possible risk of infection, have not been detailed due to their low economic value and the difficulty in carrying out surveys. The role of wild rats as the zoonotic reservoir of *Cryptosporidium* and *Giardia* species has not been examined so far in Punjab, India, especially in the context of urban areas. In view of the above facts the present study was undertaken.

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How to cite this article: Brar, S.K., Singla, N., Singla, L.D. (2021). Concurrent infection of *Cryptosporidium* and *Giardia* in synanthropic rodents: First report from Punjab, India. Ind J Vet Sci and Biotech, 17(1): 45-47.

Source of support: Nil

Conflict of interest: None.

Submitted: 23/11/2020 **Accepted:** 23/02/2021 **Published:** 25/03/2021

Materials and Methods

Collection of animals

A total of 65 rodents were live trapped from different urban areas including residences/shops (street food shops, flour mills, grocery shops etc.), poultry farms and fish market in Ludhiana city of Punjab, India using baited single- and multi-catch rat traps. In the laboratory, rodent species were identified (Singla *et al*., 2015) and kept individually in laboratory cages for faecal examination. Approval from Institutional Animal Ethics Committee for use of animals was obtained vide memo no. IAEC/2018/1153-1188 under protocol no.GADVASU/2018/IAEC/46/16.

Coproscopic examination

In the present study, formaldehyde-ether sedimentation technique and modified kinyoun acid fast stain was used to detect the parasites (Gupta and Singla 2012; Brar *et al.,* 2017). A minimum of 1 g of faecal sample was collected from each individual rodent. This procedure is especially suitable for the identification of oocysts of *Cryptosporidium*.

Results and Discussion

Out of the total 65 synanthropic rodents of two species *i.e.* the house rat, *Rattus rattus* (n=40) and the bandicoot rat, *Bandicota bengalensis* (n=25) collected from different study locations (Table 1), faecal samples of 32.30% were found infected with oocysts of *Cryptosporidium* with more prevalence in *B. bengalensis* (36%) than *R. rattus* (30%). A total of 3 (4.61%) rodents were found infected with precysts/cysts of *Giardia* spp. in concurrence with *Crytosporidium* (Table 1).

The oocysts were visible in faecal samples of rats stained uniformly as pinkish-red colour against bluish background in acid-fast stain (Fig.1A, B). Morpho-metrically, the oocysts were thick walled and oval in shape with an average size of 7.70- 9.8 x 5.5-7.0 µm resembling *C. muris*. Risk assessment of *C. muris* revealed relatively higher risk (1.20) of infection in *B. bengalensis* compared to the risk in *R. rattus*. Whereas, relative risk of infection of *Giardia* spp. was higher (1.25) in *R. rattus* than the risk on *B. bengalensis* (Table 1).

Precysts/cysts of *Giardia* spp. were seen in fresh (Fig. 1B) as well as stained slides (Fig. 1C) of faecal samples of some rats in concurrence with *C. muris* infection. Cysts of *Giardia* spp. observed in stained faecal samples were broadly ovoid and possessed two large and broadly oval nuclei and adhesive disk (Fig. 1D) whose length overlapped one half of the body length.

GarciaLivia *et al.* (2020) also reported 12.3% prevalence of *Cryptosporidium* spp. in wild rodents in Canary Islands, Spain. Torres *et al.* (2000) detected *Cryptosporidium* involved in the infection of small mammals of Spain and found mixed infection of *C. glareolus, C. parvum* and *C. muris* in rodents. Gholipoury *et al.* (2016) surveyed wild rats of Turkmen Sahra, Iran and found 6.6% infection of *Cryptosporidium* spp. *Cryptosporidium* species, including *C. muris*, were identified in humans, particularly in immuno-compromised individuals (Chappell *et al.,* 2015). *C. muris* was first identified in the gastric glands of mice. But in humans, the first reported case of *C. muris* was published in 2000 (Chappell *et al.,* 2015), and since that time, numerous additional *C. muris* cases have been reported in the literature (Lv *et al.,* 2009; Chappell *et al.,* 2015).

Giardiosis is a neglected parasitic disease affecting the physical and mental development of children, especially those in developing countries (Eppig et al., 2010). More than 280 million human infections are estimated by the WHO per year in Africa, Asia and America (Martínez-Gordillo et al., 2014). As observed in the acid fast stained faecal smears microscopically in the present study, there are several reports on the prevalence of *Giardia* spp. in wild and laboratory rodents (Seifollahi *et al.,* 2016; Helmy *et al.*, 2018; Mohaghegh *et al.,* 2018).

Concurrent infection of *Cryptosporidium* and *Giardia* has been reported in calves on two Ohio farms and humans in Africa

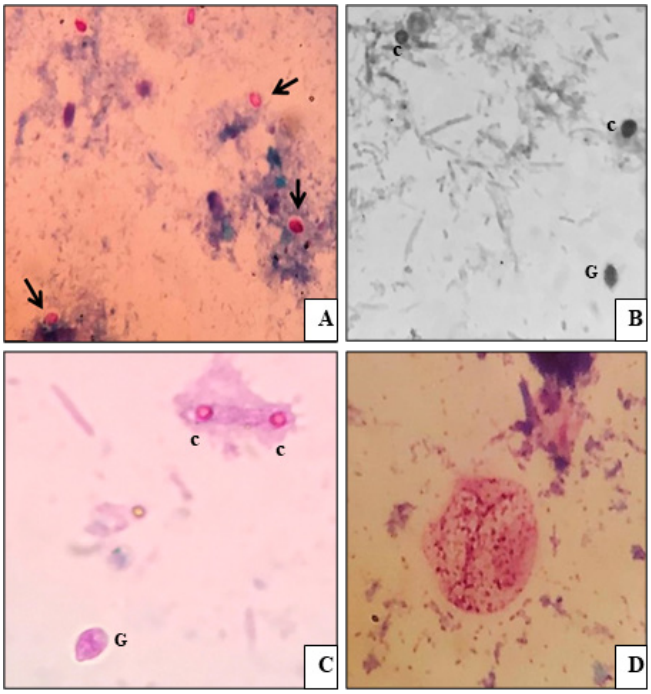

Fig. 1: A. Acid fast stained oocysts (arrows) of *Cryptosporidium muris* at 40x, B. Concurrent *Giardia* cyst (G) and *Cryptosporidium muris* (C) oocyst infection after formaldehyde–ether sedimentation at 40x, C. Concurrent *Giardia* cyst (G) and *Cryptosporidium muris* (c) oocyst at 40x and D. Acid fast stained *Giardia* cyst at 100x.

Table 1: Prevalence and risk assessment of *Cryptosporidium* and *Giardia* infections in synanthropic rodents.

Protozoan parasites found	*Rodents species*	*Host examined*	*Host Infected*	*Percent infected*	*Relative risk*	*95% Confidence interval*	
						Lower limit	*Upper limit*
Cryptosporidium muris	*Rattus rattus*	40	12	30.00	1.00	0.51	1.95
	Bandicota bengalensis	25	9	36.00	1.20	0.59	2.42
	Overall	65	21	32.30	-	-	-
Giardia sp. + *Cryptosporidium muris*	*Rattus rattus*	40	2	5.00	1.25	0.11	13.07
	Bandicota bengalensis	25	1	4.00	1.00	0.06	15.11
	Overall	65	3	4.61	-	-	-

(Squire and Ryan, 2017). Transmission of these two parasites from rodents to humans and other animals can occur directly through accidental ingestion of oocysts/cysts excreted in faeces (Daniels *et al.,* 2015) and indirectly by consumption of contaminated food and water (Pumipuntu and Piratae, 2018).

The present study is the first record of coproscopical analysis to determine prevalence of *C. muris* alone and in concurrence with *Giardia* sp. from urban synanthropic rodents of Punjab, India. The study creates awareness about the role of rodents as reservoirs of protozoan parasites and the possible modes of transmission thereby suggesting proper management of rodent pests and avoidance of contamination caused by them.

Acknowledgement

Authors are thankful to the Indian Council of Agricultural Research, New Delhi, India for providing funds in the form of All India Network Project on Vertebrate Pest Management (Rodent Control) at Department of Zoology, Punjab Agricultural University, Ludhiana, India.

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