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# Diversity, Prevalence and Risk Assessment of Nematode Parasites in *Tatera indica* found in Punjab State

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## ABSTRACT

**Background:** Present study was aimed to record diversity, prevalence and risk assessment of nematode parasites in Indian gerbil, *Tatera indica,* a wild rodent species found in crop fields.

**Methods:** Total 180 Indian gerbils (*T. indica*) were live trapped from crop fields of Ludhiana, Punjab between April, 2019 to March, 2020 and examined for the diversity and prevalence of nematode parasites in liver and gastrointestinal tract.

**Result:** Only 33 (18.33%) gerbils were found infected with four nematodes species *i.e. Nippostrongylus brasiliensis* (39.40%), *Calodium hepaticum* (21.21%), *Trichuris muris* (21.21%) and *Syphacia muris* (18.18%). Eggs of *N. brasiliensis*, *T. muris* and *S. muris* were found in faecal samples. Pale yellow lesions on surface of liver and subsequent histopathology of infected liver indicated the presence of *C. hepaticum* eggs entrapped in parenchyma. Host age ($\chi^2$ = 8.78, P = 0.03) and season ($\chi^2$ = 9.21, P = 0.01) significantly affected the prevalence of *N. brassiliensis* and *C. hepaticum*, respectively. Shannon-Weiner index and Species Evenness of nematode parasites were maximum in monsoon season (1.04 and 0.94, respectively). Based on results proper rodent pest management is advised by integrated control measures at village level to prevent spread of diseases caused by them.

**Key words:** Endoparasites, Nematodes, Risk assessment, *Tatera indica,* Zoonosis.

## INRODUCTION

Rodents are a big nuisance for mankind by being major household and agricultural pests (Singla and Babbar, 2010). Beside this, they are reservoirs of parasites of veterinary and public health importance resulting in socioeconomic and public health issues (Singla *et al.* 2008; Nateghpour *et al.* 2014; Singla *et al.* 2016). Disease transmission through rodents follows both direct and indirect routes (Singla *et al.* 2008; Ranjbar *et al.* 2017), by coming in contact with rodent excrements, through contaminated food, water and through arthropod vectors (Meerburg, 2009). Reports from Iran, Korea, Singapore and various other countries have indicated the presence of different nematode species in rodents *(*Pakdel *et al.* 2013*;* Kim *et al.* 2015*;* Mendenhall *et al*. 2017, respectively*).*

The Indian gerbil, *Tatera indica* is a wild rodent species belonging to family Muridae and subfamily Gerbillinae found in dry land crop fields, wastelands and near human establishments in arid and sub-humid habitats (Singla *et al.* 2017). It has also been found infested with many species of helminthes (Singla *et al.* 2008). Cosmopolitan nature and high reproductive rate of rodents is responsible for wide spread of diseases (Ogolla *et al.* 2019). Rodent borne zoonotic diseases are threat to public health and have emerged as epidemic from time to time for instance; capilliarisis caused by genus *Calodium* infected more than 1300 persons in 1967-68 and 1978-79 and 90 persons died in Philippine (Bair *et al.* 2004). Very few workers have set forth the regional studies on the occurrence of nematodes of *T. indica*. Considering this in view, the present study was conducted on nematodes of the Indian gerbil, *T. Indica* to know their diversity, prevalence and risk assessment.

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## MATERIALS AND METHODS

### Collection and maintenance of animals

The Indian gerbil, *T. indica* (n = 180) were collected from the crop fields of village Barewal Dogra, district Ludhiana of Punjab (India) using single catch live traps from April, 2019 to March, 2020 and brought to the rodent laboratory at Department of Zoology, Punjab Agricultural University, Ludhiana. The whole period was divided into three seasons *i.e.* summer (March-June), monsoon (July-October) and winter (November-February) with 60 gerbils collected in each season. Male and female gerbils were identified based on the presence of scrotum and vaginal opening, respectively, whereas young and mature gerbils were categorised on the basis of scrotal testes, perforated or imperforated vagina and body weight. Approval for use of animals was obtained from Institutional Animal Ethics Committee (Memo no. IAEC/ 2019/118-221 protocol no. GADVASU/2019/IAEC/51/17).

**Collection and identification of parasites**

The gerbils were sacrificed using over dose of ketamine-xylazine combination (>75 + 10 mg/kg body weight i/p), as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines 2018 and dissected via mid ventral incision to record the presence of endoparasites in the visceral organs. The content of gastrointestinal tract was transferred into a petri dish containing normal saline solution and examined both with the naked eye and under a dissecting microscope. The nematode parasites collected were transferred to 70% ethanol for further identification (Taylor *et al.* 2016). The data obtained was used to determine the following parameters:

$$\text{Percent host infected} = \frac{\text{Number of hosts infected}}{\text{Number of hosts examined}} \times 100$$

$$\text{Mean parasitic intensity} = \frac{\text{Number of parasites found}}{\text{Number of hosts infected}}$$

$$\text{Mean parasitic abundance} = \frac{\text{Number of parasites found}}{\text{Number of hosts examined}}$$

**Faecal examination and histopathology**

Faecal floatation examination and micrometry of eggs was conducted to determine the presence and identification of eggs (Taylor *et al.,* 2016). The length and width of different eggs were recorded as per the method described (Soulsby, 1982; Bowman, 1999). The histopathology of infected liver was performed as per Luna (1968).

**Statistical analyses**

The data was analysed using Pearson's Chi-square test at 5% level of significance by logistic analysis using SAS 9.4 software. The relative risk was determined as per the method described in Thrusfield (2005). Community characteristics such as species richness, species evenness and Shannon-Weiner index for different nematode species were also determined (McIntosh, 1967; Magurran, 2004; Ortiz-Burgos, 2016) as per the formulae given below:

$$\text{Shannon-Wiener Index (H)} = \sum[(pi)\times\ln(pi)]$$

Where,

pi = proportion of total sample represented by species i (obtained by dividing number of individuals of species i by total number of samples).

Species richness (S) = number of species.

Species evenness = H/Hmax.

Where, Hmax = ln(S).

## RESULTS AND DISCUSSION

**Overall parasitic prevalence**

Out of the total 180 *T. indica* consisting of 79 males, 101 females, 58 mature and 122 immature ones trapped from crop fields, 33 (18.33%) were found infected with four species of nematode parasites *viz. Syphacia muris*, *Trichuris muris*, *Nippostrongylus brasiliensis* and *Calodium hepticum*. Maximum numbers of gerbils (13, 39.40%) were infected with *N. brasiliensis* followed by *T. muris* (7, 21.21%), *C. hepticum* (7, 21.21%) and *S. muris* (6, 18.18%). Out of total 173 nematode parasites found in the intestine of *T. indica*, maximum number of parasites were of *S. muris* (134, 77.46%) followed by *N. brasiliensis* (25, 14.45%) and *T. muris* (14, 8.09%). Developmental stages of *C. hepticum* were found infecting the liver. In contrast to present study, Pakdel *et al.* (2013) found comparatively less infestation of *C. hepaticum* (3.62%), *T. muris* (14.49%) and *S. muris* (2.89%) in wild rodent species. They did not report *N. brassiliensis* infection. Harandi *et al.* (2014), however, found 40% gerbils infected with nematodes in Iran. This variation can be due to the differences in climatic conditions of different study areas. In accordance with our findings, Kataranovski *et al.* (2011) observed infection of *N. brassiliensis*, *C. hepaticum*, *T. muris* and *S. muris* alongwith *Heterakis spumosa* in Norway rat, *Rattus norvegicus*. Recently, Mohtasebi *et al.* (2020) found wild rodent species, *Dryomys nitedula* infected with *T. muris* and a species of genus *Syphacia* alongwith other helminthes. Arzamani *et al*. (2017) identified *T. trichuris* and *N. brassiliensis* along with *Syphacia oblevata* in rodents inhabiting Iran.

**Morphometric analysis of eggs found in faecal samples**

Faecal examination revealed the presence of eggs of three nematode species *i.e. S. muris, N. brasiliensis* and *T. muris.* The eggs of *S. muris* were 72.50-82.25µm long and 22.00-36.50 wide. These were asymmetrical, slightly flattened on one side and curved on the other side (Fig 1a). The eggs of *T. muris* were 67.50-70.75 µm in length and 35.00-40.50 µmin width having plugs at both the poles giving them acharacteristic bipolar shape (Fig 1b). The eggs of *N. brasiliensis* were comparatively small and ellipsoidal inshape. They had a thin shell and measured 50.00-70.00 µmin length and 27.00-40.00 µm in width (Fig 1c). Eggs *C. hepaticum* were not found in faeces but histopathology of infected liver revealed the presence of eggs entrapped in liver parenchyma. These were 55.00-72.50 µm in length and 32.50-40.00 µm in width. The parenchyma showed granular degeneration, necrosis and microgranulomas (Fig 1d). In contrary to present study, Sharma *et al.* (2013) observed eggs of *C. hepaticum* in faecal samples along with eggs of *S. muris, T. muris* and other strongyle eggs.

**Morphology and morphometery of adult worms**

Length of the adult worm of *S. muris* inhabiting the ceacum of *T. indica* varied from 1.20-3.40 mm. They had characteristic oesophageal bulb at the anterior region (Fig 2a). *T. muris* was a small nematode of 10.00-20.00 mm length with cylindrical non-segmented body in caecum (Fig 2b, c). *N. brasiliensis* (10.50-20.00 mm in length) was found to inhabit small intestine and ceacum. The males were having characteristic bursa at the posterior end (Fig 2d). *C. hepaticum* infection was found in the liver of *T. indica* in the form of pale yellowish lesions on the surface of liver (Fig 3).

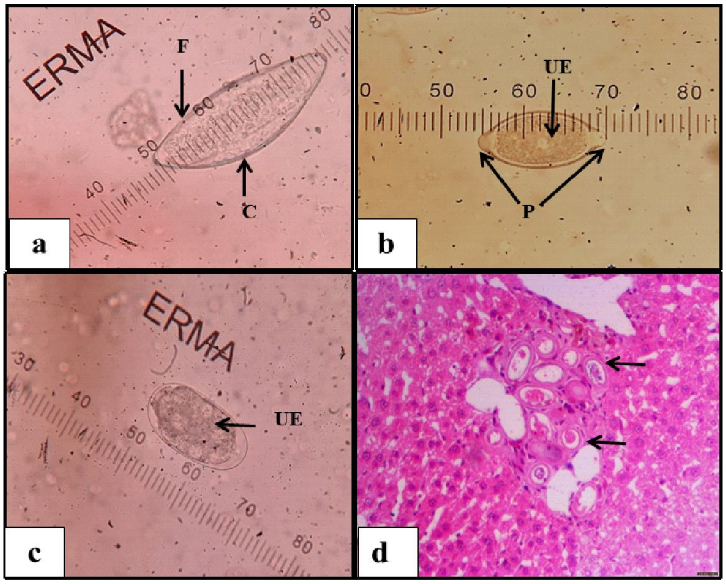


**Fig 1: a)** Egg of *Syphacia muris* at 400x with one side flattended (F) and other curved (C), **b)** Egg of *Trichuris muris* at 400x with polar plugs (P) on two ends and undifferentiated embryo (UE) inside, **c)** Egg of *Nippostrongylus brassiliensis* at 400x with undifferentiated embryo (UE) inside and **d)** Eggs of *Calodium hepaticum* enclosed in liver parenchyma at 400x (arrows).

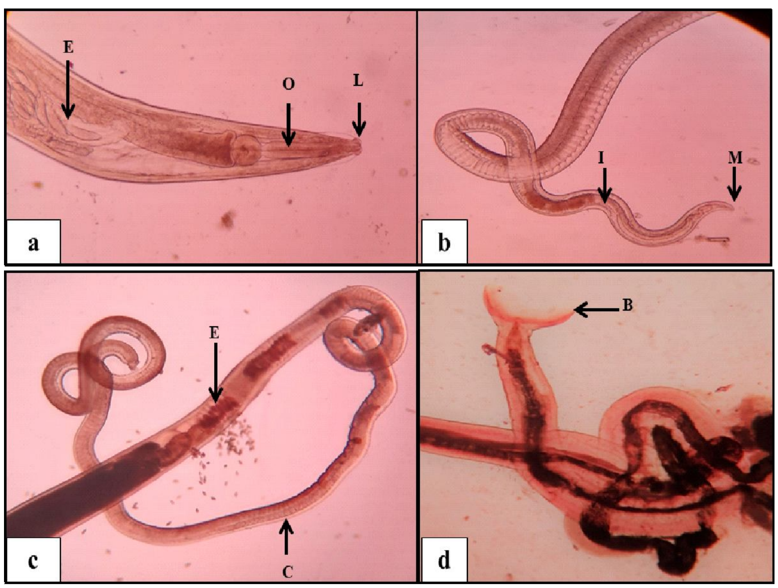


**Fig 2: a)** Anterior part of female *Syphacia muris* showing lips (L), oesophagus (O) and eggs (E), **b)** Anterior part of *Trichuris muris* showing mouth (M) and intestine (I), **c)** Posterior part of female *Trichuris muris* showing cuticular lining (C) and eggs (E) and **d)** posterior part of male *Nippostrongylus brassiliensis* showing bursa (B).

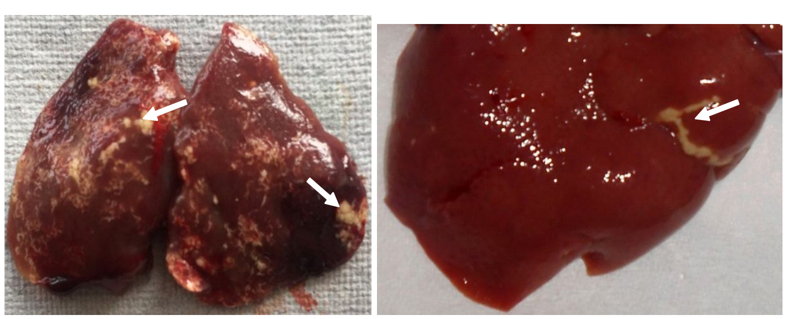

**Fig 3:** Liver of *T. indica* infected with *C. hepaticum* (arrows showing pale yellow lesions).

### Community characteristics

Shannon-Weiner index and Species Evenness of different nematode species found in *T. indica* were maximum in monsoon season (1.04 and 0.94, respectively) followed by winter (0.80 and 0.72, respectively) and summer seasons (0.45 and 0.40, respectively). Also these indices were more in female (0.77 and 0.70, respectively) and mature (1.01 and 0.91, respectively) gerbils as compared to male (0.70 and 0.63, respectively) and immature (0.43 and 0.39, respectively) gerbils. In all, nematode parasites were more diverse and evenly distributed in monsoon season as compared to summer and winter seasons. Overall Shannon-Weiner index (0.74) and Species Evenness (0.67) for nematode parasites were low in present study indicating less equitable distribution and abundance of species.

### Percent infection and risk assessment

Total six (3.33%) gerbils were found infected with *S. muris.* Higher infection rate was found in immature (4.10%) and male (5.06%) gerbils during summer season (6.67%). However, statistically, season ($\chi^2$ = 3.10, P = 0.21 and df = 2), age ($\chi^2$ = 0.68, P = 0.40 and df = 1) and sex ($\chi^2$ = 1.3, P = 0.25 and df = 1) had no significant effect on the infectivity of *S. muris*. Mean parasitic intensity and abundance were also more in summer season (24.75 and 1.65) in immature gerbils (23.60 and 0.96) indicating higher chances of infection of *S. muris* from immature gerbils during summer season. The mean parasitic intensity was more in female (27.50) gerbils, while the mean parasitic abundance was more in male (1.00) gerbils. The relative risk of occurrence of *S. muris* infection was more in summer season (3.99) through immature (2.38) and male gerbils (2.55) (Table 1).

Total seven (3.89%) gerbils were found infected with *T. muris.* Higher rate of infection was observed in mature (5.17%) and female (4.95%) gerbils in summer season (3.33%). Mean parasite intensity was higher in male gerbils (4.50), while it was similar across the seasons and age groups. Mean parasite abundance of *T. muris* was more during monsoon season in mature male gerbils (Table 1). Mature (1.58) and female (1.96) gerbils had relatively higher risk associated with them with respect to transmission of *T. muris* infection in summer season (3.33) (Table 1). But, statistically, the season ($\chi^2$ = 0.29, P = 0.86 and df = 2), host age ($\chi^2$ = 0.37, P = 0.53 and df = 1) and sex ($\chi^2$ = 0.69, P = 0.40 and df = 1) had no significant effect on *T. muris* infection.

**Table 1:** Prevalence and risk assessment of different nematode parasites.

| Nematode species | Epidemiological factors | | Animals examined (n=180) | Animals infected (%) | Number of parasites | Mean intensity | Mean abundance | Chi square value | P value (d.f.) | Relative risk |
|---|---|---|---|---|---|---|---|---|---|---|
| *Syphacia muris* | Season | Summer | 60 | 4 (6.67) | 99 | 24.75 | 1.65 | 3.10 | 0.21 | 3.99 |
| | | Monsoon | 60 | 1 (1.67) | 15 | 15.00 | 0.25 | | (2) | 1.00 |
| | | Winter | 60 | 1 (1.67) | 20 | 20.00 | 0.33 | | | 1.00 |
| | Age | Mature | 58 | 1 (1.72) | 16 | 16.00 | 0.27 | 0.68 | 0.40 | 1.00 |
| | | Immature | 122 | 5 (4.10) | 118 | 23.60 | 0.96 | | (1) | 2.38 |
| | Sex | Male | 79 | 4 (5.06) | 79 | 19.75 | 1.00 | 1.30 | 0.25 | 2.55 |
| | | Female | 101 | 2 (1.98) | 55 | 27.50 | 0.54 | | (1) | 1.00 |
| *Trichuris muris* | Season | Summer | 60 | 2 (3.33) | 4 | 2.00 | 0.06 | 0.29 | 0.86 | 3.33 |
| | | Monsoon | 60 | 3 (1.00) | 6 | 2.00 | 0.10 | | (2) | 1.00 |
| | | Winter | 60 | 2 (1.67) | 4 | 2.00 | 0.06 | | | 1.67 |
| | Age | Mature | 58 | 3 (5.17) | 6 | 2.00 | 0.10 | 0.37 | 0.53 | 1.58 |
| | | Immature | 122 | 4 (3.28) | 8 | 2.00 | 0.07 | | (1) | 1.00 |
| | Sex | Male | 79 | 2 (2.53) | 9 | 4.50 | 0.11 | 0.69 | 0.40 | 1.00 |
| | | Female | 101 | 5 (4.95) | 5 | 1.00 | 0.05 | | (1) | 1.96 |
| *Nippostrongylus brassiliensis* | Season | Summer | 60 | 5 (8.33) | 10 | 2.00 | 0.16 | 0.66 | 0.71 | 1.67 |
| | | Monsoon | 60 | 5 (8.33) | 11 | 2.20 | 0.18 | | (2) | 1.67 |
| | | Winter | 60 | 3 (5.00) | 4 | 1.33 | 0.06 | | | 1.00 |
| | Age | Mature | 58 | 9 (15.52) | 18 | 2.00 | 0.31 | 8.78 | 0.003 | 4.73 |
| | | Immature | 122 | 4 (3.28) | 7 | 1.75 | 0.06 | | (1) | 1.00 |
| | Sex | Male | 79 | 8 (10.13) | 15 | 1.87 | 0.18 | 1.77 | 0.18 | 2.05 |
| | | Female | 101 | 5 (4.95) | 10 | 2.00 | 0.10 | | (1) | 1.00 |
| *Calodium hepaticum* | Season | Summer | 60 | 6 (10.00) | - | - | - | 9.21 | 0.01 | 5.99 |
| | | Monsoon | 60 | 1 (1.67) | - | - | - | | (2) | 1.00 |
| | | Winter | 60 | 0 (0.00) | - | - | - | | | - |
| | Age | Mature | 58 | 1 (1.67) | - | - | - | 1.07 | 0.30 | 1.00 |
| | | Immature | 122 | 6 (4.92) | - | - | - | | (1) | 2.95 |
| | Sex | Male | 79 | 2 (2.53) | - | - | - | 0.69 | 0.40 | 1.00 |
| | | Female | 101 | 5 (4.95) | - | - | - | | (1) | 1.96 |

Total thirteen (7.22%) gerbils were found infected with *N. brassiliensis* with higher rate of infection in mature (15.52%) and male (10.13%) gerbils in monsoon and summer seasons (8.33% each). Statistically, the infestation of *N. brassiliensis* was not found to be affected by season ($\chi^2$ = 0.66, P = 0.71 and df = 2) and sex ($\chi^2$ = 1.77, P = 0.18 and df = 1) of the host. But age of the host significantly affected rate of *N. brassiliensis* infection ($\chi^2$ = 8.78, P = 0.003 and df = 1). Mean parasitic intensity and abundance were highest in monsoon season (2.20 and 0.18, respectively). Mean parasitic infestation was higher in mature (0.31) male (0.18) gerbils.

Liver of seven (3.89%) gerbils was found infected with *C. hepaticum* with higher infection in immature (4.92%) and female gerbils (4.95%) during summer season (10.00%). Statistically, it was observed that season had direct impact on the infestation of this nematode ($\chi^2$ = 9.21, P = 0.01 and df = 2). No significant difference was, however, observed for host age ($\chi^2$ = 1.07, P = 0.30 and df = 1) and sex ($\chi^2$ = 0.69, P = 0.40 and df = 1). Relatively higher risk of *C. hepaticum* infection was found associated with immature (2.50) and female (1.96) gerbils during summer season (5.99). Liver of infected animals was found containing numerous eggs of this parasite, however, the adult parasites could not be retrieved. No *C. hepaticum* infection was observed in winter season. In contrast to our study, Kataranovski *et al.* (2011) observed *Capillaria* sp. infection in rodents in winter season. Although rare, *C. hepaticum* is responsible for hepatic capillariosis in humans from different parts of the world (Fuehrer 2014).

Similar to present study, Moudgil *et al.* (2018) gave morpho-pathological description of fatal concurrent intestinal and renal parasitism in *Columba livia domestica* in India**.** In present study, a significant effect was observed of host age on *N. brassiliensis* infection (being higher in mature gerbils) and season (being higher in summer season) on *C. hepaticum* infection. This finding was seconded by the study of Hernandez *et al.* (2020) conducted in Southern Guatemala who observed higher infection of *Nippostrongylus* sp. in mature male rodents. However, Kataranovski *et al.* (2011) did not find effect of host age on any of the nematode infections. Rather they found significant affect of sex on the prevalence of *T. muris* and *C. hepaticum*. Coomansingh *et al.* (2009) reported that *N. brasiliensis* infection in adult rodents was much more than any other endoparasite infection which is in agreement with our present study. With *N. brasiliensis* infection, there is T-cell mediated immune response stimulation in the host resulting in expulsion of worms, however, this phenomenon fails to develop when infection occurs in rats having age less than 6 weeks and thus worms persist into adult life (Wakelin 1996). This could be the reason why *N. brasiliensis* infection was more in mature gerbils. Overall, in present study, higher nematode infection was observed in summer season as compared to winter and monsoon season as also observed by Kataranovski *et al.* (2011) which may be due to more foraging activity of rodents in summers thereby increasing their vulnerability to parasites (Eccard and Herde 2013; Tijjani *et al.* 2020). On the other hand, higher prevalence of nematode parasites was observed in sheep during winter and monsoon than in summer season by Pazhanivel *et al*. (2004). Pawar *et al*. (2020) have also reported higher nematode infestation (70.58%) in captive wild animals. The presence of these nematode parasites in gerbils suggests the potential risk of rodent-borne disease transmission.

## CONCLUSION

The present study indicated *T. indica* to be risk associated as being loaded with nematode parasites of veterinary and public health significance. Based on present study proper rodent pest management is advised using integrated control measures at village level in crop fields to avoid damage to crops and prevent spread of diseases caused by them.

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