POTENTIAL OF *Bandicota bengalensis* Gray and Hardwicke INHABITING COMMENSAL AREAS IN TRANSMITTING PARASITIC ZOONOTIC DISEASES

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirement
for the degree of

INTEGRATED MASTER OF SCIENCE (HONS.)
in
ZOOLOGY
(Minor Subject: Entomology)

By

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CERTIFICATE I

This is to certify that the thesis entitled, **"Potential of *Bandicota bengalensis* Gray and Hardwicke inhabiting commensal areas in transmitting parasitic zoonotic diseases"** submitted for the degree of **Integrated Master of Science (Hons.)**, in the subject of **Zoology** (Minor subject: **Entomology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ms. Shivani Rara (L-2013/14-BS-86-IM)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled, "**Potential of *Bandicota bengalensis* Gray and Hardwicke inhabiting commensal areas in transmitting parasitic zoonotic diseases**" submitted by **Ms. Shivani Rara** (**L-2013/14-BS-86-IM**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Integrated Master of Science (Hons.)** in the subject of **Zoology** (Minor subject: **Entomology**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

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ABSTRACT

Present study recorded the potential of *Bandicota bengalensis* inhabiting commensal areas in transmitting parasitic zoonotic diseases. A total of 100 rats collected from fish market and railway station at Ludhiana, Punjab from November 2020 to October 2021 were examined for the presence of ecto and endoparasites along with risk factor analysis. Parasites found were identified based on morphological features of adults and their eggs. 25.00% rats collected two locations were found infected with one species of ectoparasites i.e. Oriental rat flea, *Xenopsylla cheopis*. Total 34 rat fleas were found in 25 rats with flea index of <1.0 indicating low risk of disease transmission. The liver and intestines of rats collected from both the locations were found infected with seven species of endoparasites, comprising two cestode species (*Hymenolepis nana* and *Cysticercus fasciolaris*) and five nematode species (*Nippostrongylus brasiliensis, Calodium hepaticum, Syphacia muris, Trichuris muris* and *Heterakis spumosa*). Rats were having concurrent infection of one or more parasites. 79.00% rats collected from two locations were found infected with endoparasites with mean parasite intensity and parasite index of 30.73 and 24.28, respectively indicating high risk of disease transmission. The host age, sex and season had no significant effect on parasite prevalence except that of *H. nana* and *S. muris* whose infection in rats collected from fish market was significantly affected by season. The present study suggests that proper rodent pest and vector management should be conducted in animal and human inhabitations to avoid the spread of zoonotic diseases caused by them.

Keywords: *Bandicota bengalensis,* Ecoparasites, Endoprasites, Prevalence, Relative risk, Zoonosis

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Signature of Major Advisor **Signature of the Student**

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ਵਰਤਮਾਨ ਅਧਿਐਨ ਪਰਜੀਵੀ ਜ਼ੂਨੋਟਿਕ ਰੋਗਾਂ ਨੂੰ ਸੰਚਾਰਿਤ ਕਰਨ ਵਿੱਚ ਜਾਨਵਰਾਂ ਅਤੇ ਮਨੁੱਖੀ ਵਸੋਂ ਵਾਲੇ ਖੇਤਰਾਂ ਵਿੱਚ ਰਹਿਣ ਵਾਲੇ ਬੈਂਡੀਕੋਟਾ ਬੇਂਗਾਲੈਂਸਿਸ ਦੀ ਸੰਭਾਵਨਾ ਨੂੰ ਦਰਜ ਕਰਦਾ ਹੈ। ਨਵੰਬਰ 2020 ਤੋਂ ਅਕਤੂਬਰ 2021 ਤੱਕ ਲੁਧਿਆਣਾ, ਪੰਜਾਬ ਵਿਖੇ ਮੱਛੀ ਮੰਡੀ ਅਤੇ ਰੇਲਵੇ ਸਟੇਸ਼ਨ ਤੋਂ ਇਕੱਠੇ ਕੀਤੇ ਕੁੱਲ 100 ਚੂਹਿਆਂ ਦੀ ਖਤਰੇ ਦੇ ਕਾਰਕਾਂ ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਦੇ ਨਾਲ ਐਕਟੋ ਅਤੇ ਐਂਡੋਪੈਰਾਸਾਈਟਸ ਦੀ ਮੌਜੂਦਗੀ ਲਈ ਜਾਂਚ ਕੀਤੀ ਗਈ। ਪਾਏ ਗਏ ਪਰਜੀਵੀਆਂ ਦੀ ਪਛਾਣ ਬਾਲਗਾਂ ਅਤੇ ਉਨ੍ਹਾਂ ਦੇ ਅੰਡੇ ਦੀਆਂ ਰੂਪ ਵਿਗਿਆਨਿਕ ਵਿਸ਼ੇਸ਼ਤਾਵਾਂ ਦੇ ਅਧਾਰ ਤੇ ਕੀਤੀ ਗਈ।ਦੋ ਸਥਾਨਾਂ ਤੋਂ ਇਕੱਠੇ ਕੀਤੇ 25% ਚੂਹੇ ਐਕਟੋਪੈਰਾਸਾਈਟਸ ਦੀ ਇੱਕ ਪ੍ਰਜਾਤੀ ਭਾਵ ਓਰੀਐਂਟਲ ਰੈਟ ਫਲੀ, ਜ਼ੇਨੋਪਸੀਲਾ ਚੇਓਪਿਸ ਨਾਲ ਸੰਕਰਮਿਤ ਪਾਏ ਗਏ। ਕੁੱਲ 25 ਸੰਕਰਮਿਤ ਚੂਹਿਆਂ ਵਿੱਚ 34 ਪਿੱਸੂ ਪਾਏ ਗਏ ਜਿਨ੍ਹਾਂ ਦਾ ਇਡੈਂਕਸ <1.0 ਸੀ ਜੋ ਬਿਮਾਰੀ ਦੇ ਸੰਚਾਰ ਦੇ ਘੱਟ ਜੋਖਮ ਨੂੰ ਦਰਸਾਉਂਦਾ ਹੈ। ਦੋਵਾਂ ਥਾਵਾਂ ਤੋਂ ਇਕੱਠੇ ਕੀਤੇ ਚੂਹਿਆਂ ਦੇ ਜਿਗਰ ਅਤੇ ਆਂਤੜਾਂ ਨੂੰ ਐਂਡੋਪੈਰਾਸਾਈਟਸ ਦੀਆਂ ਸੱਤ ਕਿਸਮਾਂ ਨਾਲ ਸੰਕਰਮਿਤ ਪਾਇਆ ਗਿਆ, ਜਿਸ ਵਿੱਚ ਦੋ ਸੇਸਟੌਡ ਸਪੀਸੀਜ਼ (ਹਾਈਮੇਨੋਲੇਪਿਸ ਨਾਨਾ ਅਤੇ ਸਿਸਟੀਸਰਕਸ ਫੈਸੀਓਲਾਰਿਸ) ਅਤੇ ਪੰਜ ਨੇਮੇਟੋਡ ਸਪੀਸੀਜ਼ (ਨਿਪੋਸਟ੍ਰੋਂਗਾਇਲਸ ਬ੍ਰਾਸੀਲੀਏਨਸਿਸ, ਕੈਲੋਡਿਅਮ ਹਿਪੈਟਿਕਮ, ਸਾਇਫੇਸ਼ਿਆ ਮੁਓਰਿਸ, ਟਰਾਇਕੁਰਿਸ ਮਉਰਿਸ ਅਤੇ ਹੈਟਰੈਕਿਸ ਸਪੁਮੋਸਾ) ਸ਼ਾਮਲ ਸਨ। ਚੂਹਿਆਂ ਨੂੰ ਇੱਕ ਜਾਂ ਇੱਕ ਤੋਂ ਵੱਧ ਪਰਜੀਵੀਆਂ ਨਾਲ ਸੰਕਰਮਿਤ ਪਾਇਆ ਗਿਆ । ਇਕੱਠੇ ਕੀਤੇ ਗਏ 79.00% ਚੂਹਿਆਂ ਨੂੰ ਕ੍ਰਮਵਾਰ 30.73 ਅਤੇ 24.28 ਦੇ ਪੈਰਾਸਾਈਟ ਤੀਬਰਤਾ ਤੇ ਪੈਰਾਸਾਈਟ ਇੰਡੈਕਸ ਨਾਲ ਸੰਕਰਮਿਤ ਪਾਇਆ ਗਿਆ, ਜੋ ਕਿ ਬਿਮਾਰੀ ਦੇ ਸੰਚਾਰ ਦੇ ਉੱਚ ਜੋਖਮ ਨੂੰ ਦਰਸਾਉਂਦਾ ਹੈ। ਮੇਜ਼ਬਾਨ ਦੀ ਉਮਰ, ਲਿੰਗ ਅਤੇ ਸੀਜ਼ਨ ਦਾ ਪਰਜੀਵੀ ਪ੍ਰਸਾਰ 'ਤੇ ਕੋਈ ਮਹੱਤਵਪੂਰਨ ਪ੍ਰਭਾਵ ਨਹੀਂ ਸੀ ਮਿਲਿਆ ਸਿਵਾਏ ਐੱਚ. ਨਾਨਾ ਅਤੇ ਐੱਸ. ਮੂਰੀਜ਼ ਜਿਨ੍ਹਾਂ ਦੇ ਮੱਛੀ ਬਾਜ਼ਾਰ ਤੋਂ ਇਕੱਠੇ ਕੀਤੇ ਚੂਹਿਆਂ ਵਿੱਚ ਸੰਕਰਮਣ ਸੀਜ਼ਨ ਦੁਆਰਾ ਮਹੱਤਵਪੂਰਨ ਤੌਰ 'ਤੇ ਪ੍ਰਭਾਵਿਤ ਹੋਇਆ ਸੀ। ਮੌਜੂਦਾ ਅਧਿਐਨ ਸੁਝਾਅ ਦਿੰਦਾ ਹੈ ਕਿ ਜਾਨਵਰਾਂ ਅਤੇ ਮਨੁੱਖੀ ਵਸੋਂ ਵਿੱਚ ਚੂਹੇ ਦੇ ਕੀੜਿਆਂ ਅਤੇ ਵੈਕਟਰਸ ਦਾ ਸਹੀ ਪ੍ਰਬੰਧਨ ਕੀਤਾ ਜਾਣਾ ਚਾਹੀਦਾ ਹੈ ਤਾਂ ਜੋ ਉਨ੍ਹਾਂ ਦੁਆਰਾ ਹੋਣ ਵਾਲੀਆਂ ਜ਼ੂਨੋਟਿਕ ਬਿਮਾਰੀਆਂ ਦੇ ਫੈਲਣ ਤੋਂ ਬਚਿਆ ਜਾ ਸਕੇ।

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CHAPTER I

INTRODUCTION

Rodents represent the largest order of living mammals. There are about 2277 known species of rodents belonging to 33 families, constituting nearly 42% of the total mammalian diversity (Singla 2021). Rodents are found in almost every habitat except Antarctica and some islands. Due to their high reproductive ability, they are widely distributed and have adapted to various environments and climates (Parshad 1999). Rodents cause direct damage to crops through feeding and indirect damage through spoiling, contaminating and hoarding during pre- and post-harvest stages. Pre-harvest rodent losses to rice and wheat crops have been estimated to be 5-15%, while the losses of grains are roughly 25-30% at post-harvest stage, resulting in a loss of at least US$ 5 billion in India (Hart 2001). Rodents exist in close proximity to human populations, farm animals and pets in many regions. Such rodents living in close association with mankind and its domestic animals are a serious threat for transmission of diseases (Meerburg *et al* 2009, Nateghpour *et al* 2012). They are considered as the reservoir hosts for at least 60 zoonotic diseases and play an important role in their transmission in different ways (Dhamana *et al* 2020). Rodents have drawn the attention of WHO experts due to their importance as a public health issue (WHO 2007). Infections such as rat bite fever, murine typhus, leptospirosis, toxoplasmosis, trichinosis, salmonellosis and plague which have killed more than 20 million people worldwide are caused by parasite, viral and bacterial agents that are transmitted to humans by rodents.

Rodents are most commonly found in the urban and peri-urban areas. Their breeding has rapidly increased due to the abundance of food resources and lack of environmental hygiene in such areas (Garedaghi and Khaki 2014). Their predatory and depredatory habits have a pronounced impact on human economies worldwide (Singleton *et al* 2010). About 217 species of rodents have been found inhabiting disease-causing agents viz. bacteria, viruses, protozoans, helminths, insects and fungi (Han *et al* 2015). Through these agents' rodents transmit diseases like plague, leptospirosis, salmonellosis, rat bite fever, leishmaniasis, trypanosomosis, babesiosis, hentavirus pulmonary syndrome, haemorrhagic fever and many more (Seifollahi *et al* 2016).

Rodents transmit diseases directly and indirectly. Direct transmission of diseases is through rat bite, consumption of food or water contaminated with faeces or urine of infected rodent or through inhaling air borne pathogens from rodent excrements (Singla *et al* 2008a, Meerburg *et al* 2009). Indirect transmission is through contact with arthropod vectors like ticks, mites, fleas, lice, flies etc. Rodents in association with arthropod ectoparasites play a key role in the spread of viral, bacterial, rickettsial, protozoal and helminthic diseases (Rasti *et al* 2001, Stojcevic *et al* 2004, Masan and Stanko 2005, Dehghani 2011). In indirect

transmission, rodents act as amplifier of the pathogen (Meerburg *et al* 2009). Increase in rodent population can ultimately lead to increased chances of spread of zoonotic diseases (Stojcevic *et al* 2004) resulting in socioeconomic and public health problems (Nateghpour *et al* 2014). In some cases, rodents do not transmit pathogens to humans themselves, but they are important as intermediate hosts in order to maintain the parasite's cycle of life (Herwig 2006).

Ectoparasites live on the animal's skin or fur. Insecta and Arachnida are the two classes of rodent ectoparasites. Lice and fleas belong to the class Insecta, while ticks and mites belong to the Arachnida. All of these parasites feed on blood. The louse is a lifelong parasite on the rodent's body, whereas ticks, mites and fleas are only present for a short time (Solanki *et al* 2013). *Tulameria, Bartonella, Rickettsia* and *Trypanosoma* were commonly transmitted disease agents by lice and mites found on rats (Rahdar *et al* 2015). Ticks in the Ixodidae family can spread tick-borne encephalitis and Crimean-Congo haemorrhagic fever from infected animals to healthy ones (Kia *et al* 2010, Zandehfili *et al* 2015). Rat fleas are plague carriers. About 20 diseases are transmitted from rodents to humans and animals by all of these blood sucking parasites (Khatoon *et al* 2004, Singla *et al* 2008a).

Although the role of rat mites as vectors in naturally occurring human infections has not been established, there are numerous publications indicating that mite, *Ornithonyssus bacoti* experimentally transmits *Rickettsia akari* (rickettsial pox), *Francisella pestis* (plague), Coxsackie virus, *Francisella tularensis* (tularemia), and *Trypanosoma cruzi* (Chagas disease). Despite their role as carriers, some ectoparasites cause severe itching, skin abrasions, hair loss, ulcerated skin, and other symptoms in the affected mammals (Nateghpour *et al* 2012). The mite, which causes rat mite dermatitis in humans, is usually found in tropical and mild climate zones (Beck and Fölster-Holst 2009).

Endoparasites live inside an animal's internal organs, blood and body cavity. Several types of endoparasites have been found to be capable of transmitting to humans. Many nations have reported zoonotic cases of these parasites. Infectious protozoans such as *Giardia muris, Toxoplasma gondii, Spironucleus muris, Eimeria muris, Hepatozoan muris, Trypanosoma lewisi, Cryptosporidium* spp., *Babesia muris* etc. parasitize rats and mice causing infections in humans (Nama and Parihar 1976, Soulsby 1982, Claveria *et al* 2005, Seifollahi *et al* 2016). Majority of the endoparasites found in rodents are helminths which are divided into nematodes, cestodes, trematodes and acanthocephalans. Depending on the intermediate host's needs, they may have a monoxenous or heteroxenous life cycle. Parasites with a monoxenous life history, directly infect the animal through ingestion or contact with contamination, while the animals with a heteroxenous life cycle, complete their larval development in an intermediate host before moving on to the final host. Endoparasites such as *Taenia taeniaeformis* accounted for the highest infection recorded (28%) followed

by *Hymenolepis nana* (19.5%) and *Calodium hepaticum* (19.1%) in Malayasia (Tiijjani *et al* 2020). Helminth parasites of different genera infected a large number of humans and livestock (Anthony *et al* 2007) and are thus important from a public health perspective (Malsawmtluangi and Tandon 2009).

Hymenolepis species are tapeworms that can be found all over the world in habitats ranging from temperate to tropical. Their eggs are passed out in rodent faeces in fields, grain stores and foodstuffs and are responsible for disease spread (Khatoon *et al* 2004). *T. taeniaeformis* is an intestinal tapeworm that has been found in domestic and wild cats, as well as in other carnivores, whereas its larval form, *Cysticercus fasciolaris*, is found in rodents that serve as intermediate hosts (Singla *et al* 2008a). Rodents are infected by ingesting the ova in contaminated food and bedding material and larvae (metacestodes) develop inside them (Jithendran and Somvanshi 1998). These metacestodes migrate to liver and develop as a chronic infection (Jithendran and Somvanshi 1998, Ekanayake *et al* 1999). *C. fasciolaris* infection is clinically asymptomatic and is considered harmless (Singla *et al* 2003). The adult worm is frequently encountered in the small intestine of the cat and wild felids (Singla *et al* 2003).

Rodents are the primary hosts of *C. hepatica* and play an important role in the spread of infection to humans and animals. *C. hepatica* adult worms inhabit the liver, and the female adult worm lays clusters of eggs in the liver parenchyma that get encapsulated as a result of chronic inflammatory reactions in the host and do not pass out in the faeces (Spratt and Singleton 2001, Mowat *et al* 2009, Singla *et al* 2013). Data about parasite life cycle, reservoirs dispersal and transmission models in each ecosystem are necessary for limitation of zoonotic parasites.

Both commensal and field rodents are the transmitters of zoonotic diseases (Singla *et al* 2008a, b, Nateghpour *et al* 2012). The lesser bandicoot rat, *Bandicota bengalensisis* Grey and Hardwicke is found in both the conditions. It is known for its aggressive behaviour and tough sound when disturbed (Meehan 1984). It is capable of adapting to all the environmental conditions and has a high reproductive rate. The natural habitat of *B. bengalensis* rats is thick forests and bushes. It has now adapted to urban areas where it gets sufficient food from sources like food storage warehouses, restaurants, markets, residences etc. (Khairuddean *et al* 2011). *B. bengalensis* acts as a reservoir of a number of parasitic infections thereby acting as a major source of zoonotic diseases (Singla *et al* 2016). Parasites affect host reproduction and other life-history traits both directly and indirectly (Richner and Heeb 1995, Kyriazakis*et al* 1996, Van Vuren 1996, Richner and Tripet 1999, Singla *et al* 2016, Warburton *et al* 2017). Indirect effects include abnormal growth and delayed sexual maturity (Scott 1988). Chronic parasitic infections lead to decreased survival and reproductive output of hosts (Feore *et al* 1997, Kristan 2002, Schwanz 2008). Knowledge of rodent parasites is of prime importance in

controlling rodent parasite-borne zoonotic diseases in the region. Systematic studies on assessing the potential of *B. bengalensis* inhabiting commensal areas in transmitting parasitic zoonotic diseases in Punjab State are limited. Present study was hence proposed with following objectives:

i) Prevalence of ectoparasites of zoonotic importance on *B. bengalensis*
ii) Prevalence of endoparasites of zoonotic importance in *B. bengalensis*

CHAPTER II

REVIEW OF LITERATURE

In addition to causing severe damage to agricultural crops, rodents play a significant role in public health, by chiefly acting as carriers or reservoirs of microbes and parasites (ecto and endoparasites) of zoonotic importance (Castillo *et al* 2003, Singla *et al* 2003, Singla *et al* 2008a, b, Meerburg 2009, Han *et al* 2015, Seifollahi *et al* 2016, Singla *et al* 2016, Dhamana *et al* 2020). Increased rodent population in an area can be directly related to the increased zoonotic diseases in human population (Bradshaw 1999, Singla *et al* 2008a). Urban rodents reside in human altered habitats and hence amplify the possibility to acquire rodent-borne zoonoses. More people have died in the last ten centuries due to rat-borne diseases than of wars. Health risks are increasing due to increase in human population density and cutting down of natural habitats which accelerate the rodent human contact. Further, situation becomes worse due to high rodent reproduction. Taylor *et al* (2001) reported that out of a total of 1415 species of infectious agents known (viruses, helminths, bacteria etc.), 868 species (61%) are of zoonotic importance. Meerburg *et al* (2009) also did risk assessment of rodent-borne diseases on public health. Zoonotic infections are carried more by urban rodents and due to urbanization, these infections are increasing rapidly. Main areas hit by these infections are developing countries which are socio-economically challenged. Various workers have studied those rodents are one of those animals that act as reservoir host of endoparasites of zoonotic importance.

2.1 Ectoparasites infesting rodents

Rodent ectoparasites are responsible for causing multiple health problems in humans and domestic animals such as anaemia, hypersensitivity, irritability and skin lesions in addition to acting as vectors of many pathogens of zoonotic importance (Moore and Gage 2005, Hanafi-Bojd *et al* 2007, Nieto *et al* 2007, Smith *et al* 2008, Amatre *et al* 2009, Fagir and El-Rayah 2009, Kia *et al* 2009, Mikhail *et al* 2010).Ectoparasites are found in the rodents in large quantity and this depends upon the species of host, their sex, age, location, geography or ecology of an area. Studies conducted on ectoparasites of rodents in the last 10 years have been reviewed here.

Fernandes *et al* (2012) conducted a study on 91 *Oligoryzomys nigripes* to investigate the effect of host's sex, body size and locality on distribution of lice. They live trapped the rats from two locations of southeast Brazil and found that males had more abundance of lice than females. Two species of lice collected were *Hoplopleura travassosi* and *Hoplopleura imparata.* Lice diversity and abundance were also affected by the locality. Herbreteau *et al* (2012) found that rodents collected from non-flooded lands, forests and paddy fields were comparatively more infested with parasites. Mihalca *et al* (2012) examined total 423 rodents

for tick parasites. Total number of ticks collected from trapped rats was 483. Seven species of ticks identified were *Ixodes ricinus, Ixodes apronophorus, Ixodes trainguliceps, Ixodes laguri, Dermacentor marginatus, Rhipicephalus sanguineus*and *Haemaphysalisaspiculata.*

Nateghpour *et al* (2012) collected 67 rodents from Baluchistan area of southeast Iran and identified ectoparasites on each species of rodents. They observed highest infestation of ectoparasites on *Tatera indica* (89.7%) followed by *Meriones hurrianae* (8.8%), *Meriones libycus* (0.75%) and *Gerbillus nanus* (0.85%). Ectoparasites present were *Xenopsylla nubica, Xenopsyllaastia, Laelaps* sp. and *Boophilus* sp. *T. indica* had highest infestation rate with an average of 34.5 ectoparasites on each animal.

Pakdad *et al* (2012) tried to find out common ectoparasites of rodents found in North of Tehran. Total 64 rats were examined, with *Rattus norvegicus* accounting for 82.8% and *Mus musculus* accounting for 17.2%. A total of 43 *R. norvegicus* individuals were infested by 1755 ectoparasites belonging to five genera and six species, including *O. bacoti* (71.7%), *Hoplopleura* spp. (17%), *Hoplopleura oenomydis* (11.3%), *Polyplax spinulosa* (3.8%), *Nosopsyllus fasciatus* (3.8%) and *I.ricinus* (1.9%). No ectoparasites were discovered from *M. musculus.*

Mazumder *et al* (2013) observed rodent flea diversity in plague endemic regions of various states of India viz. Kolar (Karnataka), Palamner (Andhra Prdadesh), Pune (Maharashtra), Surat (Gujarat), Rohru and DodraKwar (Himachal Pradesh) and Chennai (Tamil Nadu). Captured rodents were combed and fleas were collected using flea suction apparatus. *X. astia* and *X. cheopis* were observed in rodents of all the regions except Dodrakwar. In Chennai, only *X. astia* was prevalent and in Gujarat, *X. cheopis* was most prevalent.

Sharma (2013) surveyed rodents (*Rattus rattus*, *Bandicota indica* and *M. musculus*) of scrub typhus affected areas of Meghalaya for ectoparasitic diversity and recovered 66 larvae of mite, *Leptotrombidium deliense* along with 62 rat fleas. Solanki *et al* (2013) trapped 50 commensal rodents to examine the arthropod ectoparasitic fauna from different city habitats in Dehradun, India. Three species of rats were captured i.e., *R. rattus* (54%), *R. norvegicus* (40%) and *M. musculus* (6%). Of all, 18% rats were found infested with arthropod parasites such as *Xenopsylla cheopis, Ixodes* spp. and *Polyplax* spp. They also showed that *R. rattus* was the most affected rodent species and that the species and sex of the rodents had no bearing on infestation.

Guernier *et al* (2014) conducted a study on 960 rodents live trapped from Reunion Island and found infestation of four flea species viz. *X. cheopis*, *Xenopsylla brasiliensis*, *Echidnophaga gallinacean* and *Leptopsylla signis*. They also stated that flea diversity was low in cool-dry season. Ogunniyi *et al* (2014) identified *X. cheopis* and a laelaptid mite as ectoparasites on 38% of the rodents collected from houses and raw sellers in Nigeria. Five

genera of helminths inhabited 58% of rats and seven genera of protozoans inhabited 96% of rats.

Sharma and Kumar (2014) studied ectoparasitic infestation rate of rodents (*B. bengalensis* and *R. rattus*) of seaport areas of Kolkata. Overall infestation rate observed was 76.5% with mites (86.6%) dominating the list followed by fleas (11.2%) and lice (0.21%). Two species of mites i.e., larval trombiculide chigger mite (*L. deliense*) and mesostigmatid mites (*Laelaps* sp.), and two species of fleas (*X. cheopis* and *Ctenocephalides felis*) were collected. The rodent ectoparasite index was 13.6 per rat. Serological examination of rodent serum was found non-reactive for plague and Scrub typhus antibodies. Tarek and DesokyAbd EL-Allem (2014) conducted study on rodents captured from agro-ecosystems in Egypt and found that rodents were infested with three species of mites (*Amerosieus* sp., *Hypoaspis koseii* and *Cheyletus zaheri*), one species of hard tick (*Amblyomma* sp.), three species of fleas (*X. cheopis, L. segnis* and *Pulex irritans*) and one species of louse (*P. spinulosa).*

Acosta and Fernandez (2015) evaluated the presence of fleas in 144 rodents of ten species caught in the Oriental Basin of Mexico. *Polygenis vazquezi, Plusaetis parus, Meringis altipecten* and *Plusaetis mathesoni* were the most abundant flea species among total 18 species found infesting 133 rodents. Chotelersak *et al* (2015) captured 283 rodents from a Bangkok shipping port, a decaying area and a fresh food market. There was a total of 591 fleas collected, all of which were *X. cheopis*. *R. norvegicus, Rattus exulans*and *R.rattus* had 16.4, 31.8 and 51.4 percent infestation, respectively. The most fleas were found in the shipping port (68.9 percent).

Han *et al* (2015) based on secondary data and several models discovered that some rat species act as hyper reservoirs, carrying 2-11 infectious agents from different species at the same time.Hornok *et al* (2015) examined 51 synanthropic rodents (37 *M. musculus* and 14 *R. norvegicus*) for ectoparasites. The parasites found were fleas, mites and lice. No ticks were found thus confirming that synanthropic rodents are seldom parasitized by ixodid ticks.

Jensen and Magnussen (2015) examined 61 *R. norvegicus* collected from 10 locations of Faroe Island for flea and lice diversity. Fleas' species collected were *N. fasciatus* and *Ctenophthalmus nobilis* with 78% and 48% abundance, respectively. One louse species collected was *P. spinulosa* with 3% abundance. Mitkova *et al* (2015) conducted a study on 615 rodents captured from southwest Slovakia to investigate the mite infestation. 2821 mites were recovered with three species of family Laelapidae and six species of family Trombiculidae. A total of 487 rodent blood samples and 345 mite samples were examined for pathogen infestation using PCR. Fourty six blood samples and 112 mite samples were PCR positive for Rickettsial DNA (*Rickettsia helvetica* and *Rickettsia monacensis*). They thus concluded that mites are the host and reservoir of Rickettsae.

Bhuyan and Nath (2016) for the first time identified seven mite samples of species *O.*

bacoti based on their morphological characteristics, from domestic and peri-domestic rodents in and around the hilly districts of Nilgiris in India. Prevalence of such parasite necessitates further investigation on monitoring and surveillance of rickettsial diseases in the locality.

Buchholz and Dick (2017) collected nine species of ectoparasites including three ixodid tick species, five species of order Siphonoptera and one species of mesostigmatid mite on seven species of small mammals. Cull *et al* (2017) examined 217 small mammals including *Apodemus flavicollis* (Yellow necked mice), *Apodemus sylvaticus* (Wood mice) and *Myodes glareolus* (Bank vole) for tick infestation and found two tick species viz. *I. ricinus* (96%) and *Ixodes trianguliceps* (3%) with highest mean tick infestation on *A. flavicollis*. Khajeh *et al* (2017) collected 681 ectoparasites belonging to six species of fleas, two species of lice, one species of mite and two species of hard ticks from different species of rodents found in Iran. The flea species were *Xenopsylla gerbilli, X. cheopis, Xenopsylla buxtoni, Xenopsylla conformis, Nosopsyllus medus* and *Amphipysylla* spp., the lice species were *Hoplopleura* spp. and *Polyplax* spp., the mite species was *O. bacoti* and tick species were *Rhipicephalus* spp. and *Hyalomma* spp. The highest ectoparasite infestation was found in *T. indica*.

Cayol *et al* (2018) studied distribution pattern of ticks and pathogen transmission through them. They trapped rodents from eight urban and eight non-urban sites from Tyvaskyla city of central Finland. *Ixodes triangiliceps* was recovered from rodents collected from all the 16 sites and *I. ricinus* was recovered from 11 sites with overall abundance of 52.2% and 19.7%, respectively. Abundance of both the species was lower in urban sites as compared to non-urban sites. Nymphal infestation was more in summers and less in autumn. Tick borne pathogen was recovered and identified as *Borrelia burgdorferi* using real time PCR.

Ishak *et al* (2018) followed the molecular approach to identify the tick species collected from rodents of three different locations in Selangor, Malaysia. Rodents studied were *R. rattus*, *Rattus tiomanicus*, *Maxomys surifer* and *Sundamys muelleri*. They amplified the sequence of mitochondrial 16S rDNA fragment which showed 99% similarity with *Ixodes granulatus*. Khosravani (2018) reported that ectoparasites can also affect the reproductive rates, ecological fitness and the dispersal pattern of their hosts.

Maaz*et al* (2018) examined 256 mice and voles in Berlin and collected a total of 5429 arthropod parasites, with an overall prevalence of 99%. *I. ricinus* was the most common parasite, with a prevalence of 56%. Razali *et al* (2018) collected small mammals including rats from Kemasul Forest Reserve in Pahang and examined them for the presence of parasites. The parasites recovered were identified both morphologically and molecularly. One mite species, *Laelaps* sp. and five tick species, *I. granulatus* (most abundant), *Dermcentor atrosignatus, R. sanguineus, Amblyomma testudinarium* and *Haemaphysalis* sp. were

recovered. They concluded that rodent environment influences parasite prevalence. Sharma *et al* (2018) found that lice (46.5%) were the predominant ectoparasites on the rats collected from the port area of Kandla, Gujrat followed by fleas. Wang *et al* (2018) reported a new species of lice, *Haplopleura villosissima* from endemic long haired rat of Australia, *Rattus villosissimus*. It was also described as new host for *P. spinulosa* in Australia.

Lareischi*et al* (2019) analyzed 97 sigmodontine rodents in North-Eastern Argentina and collected total 835 ectoparasites including 782 mites, 50 fleas and two ticks. Out of 10 ectoparasite species identified, five were collected from a unique host species. Mustapha *et al* (2019) examined 89 wild rats and found eight different species of ectoparasites comprising *Laelaps echidnanus, Laelaps nuttalli, O. bacoti, I. granulatus, Heamaphysalis* sp., *P. spinolosa, Hoplopeura pacifica* and *X. cheopis*. About 55% of the rodents trapped were positive for at least one species of ectoparasite. About 45.8% male, 30.8% female, 42.9% adult and 32.2% juvenile rats were positive for ectoparasites of at least one species. Thille *et al* (2019) collected over 2000 ectoparasites from 88.7% of the rats (168) trapped. Ectoparasites identified were mites (on 84.6% of infested rats), lice (6.7%), fleas (3.4%) and an unidentified larval tick (0.7%). Male rats had marginally high infestation rate (83/89; 93.3%) compared to females (66/79; 83.5%). Adult rats had higher infestation rate of 90.7% (97/107) compared to juvenile rats (66.7%, 14/21).

Brar *et al* (2020) conducted faecal sample analysis of 65 synanthropic rodents of two species i.e., *R. rattus* (n=40) and *B. bengalensis* (n=25) collected from domestic and per-domestic areas for the presence of protozoan parasites using acid-fast staining. Oocysts of *Cryptosporidium* sp. were detected in 36% and 30% *B. bengalensis* and *R. rattus*, respectively. Precysts/cysts of *Giardia* sp. were also identified in 4.61% of the stained faecal smears. Krawezyk *et al* (2020) observe a positive effect of rodent diversity in natural woodlands on tick population and tick-borne pathogens prevalence. Mohammadi *et al* (2020) examined a total of 208 rodents trapped from three districts in western Iran and found only 56 (26.9%) infested with 22 species of ectoparasites. Total 312 ectoparasites were isolated including 12 flea species (54.5%), six mite species (27.3%), three tick species (13.6%), and one louse species (4.6%). Five species of fleas were recorded for the first time in Kurdistan Province including *Ctenophthalmus iranuspersicus, Paraceras melis, Nasopsyllu siranus, Paraceras* sp., and *Ctenophyllus* spp.

Islam *et al* (2021) conducted a systematic review of the available knowledge regarding ectoparasites detected on rodents in Middle Eastern countries. Following a systematic search in Pubmed, Scopus, and Web of Science, a total of 113 published articles on rodent ectoparasites were studied and analyzed. A total of 87 rodent species were documented to have ectoparasitic infestation among which *M. musculus, R. norvegicus* and *R. rattus* were the most common. Most of the articles (87 articles) reported flea infestation

followed by mites (53), ticks (44) and lice (25). *X.cheopis, P. spinulosa, O.bacoti,* and *Hyalomm arhipicephaloides* were the most commonly described flea, louse, mite and tick species, respectively. Based on the reviewed articles, the median flea, louse, mite, and tick indices were highest in Israel (4.15), Egypt (1.39), Egypt (1.27), and Saudi Arabia (1.17), respectively.

Obiegala *et al* (2021) found 90% of the small mammals infested with ectoparasites with an average of 7.3 specimens per host. Up to six species of ectoparasites were found infesting the hosts simultaneously. In total, 12 flea, 11 mite and three tick species were detected. Ticks were more prevalent than fleas or mites, with >80% of the hosts in urban and forest areas hosting ticks followed by fleas (60%) and mites (20-40%).

Samuel *et al* (2021) examined rodent species such as *R. rattus, R. norvegicus* and *M. musculus* in addition to a shrew species, *Suncus murinus* for the presence of chigger vectors of scrub typhus. The chiggers, *L. deliense* and *Schoengastiella ligula,* both known as vectors for the transmission of *Orientia tsutsugamushi* in India, were recorded in this study with prevalence of 40%. In addition, a tick (*R. sanguineus)* vector of Indian tick typhus and rat flea (*X. cheopis*) vector of plague were also reported.

2.2 Endoparasites inhabiting rodents

Endoparasites have also been found to transmit diseases to humans and animals. Various workers in the past have studied that rodents are one of those animals that act as reservoir host of endoparasites of zoonotic importance (Milazzao *et al* 2003, Asgari *et al* 2006, Waugh *et al* 2006, Rogan *et al* 2007, Gomez Villafane *et al* 2008, Singla *et al* 2008a, Coomansingh *et al* 2009, Goswami *et al* 2009, Fagir and El-Rayah 2009, Khanum *et al* 2009, Malsawmthuangi and Tandon 2009, Paramasvaran *et al* 2009, Rafique *et al* 2009, Reperant*et al* 2009). Studies conducted on ectoparasites of rodents in the last 12 years have been reviewed here.

Antoniou *et al* (2010) monitored rodents (*R. rattus* and *R. norvegicus*) in 51 different areas of Cpyrusfor re-emergence of *Echinococcus granulosus* and the presence of other endoparasites. Among 92 of the infested rats, three helminth species were found for the first time i.e., *C. fasciolaris, Hymenolepis diminuta* and *Physaloptera* spp. Kataranovski *et al* (2010) for the first time recorded the prevalence of *C. hepaticum* (10.9%) and*T. Taeniaeformis* (29.9%) infection in rodents of Europe. Kia *et al* (2010) autopsied 117 rodents including *Meriones persicus* and *Microtus socialis* species and found them infected with *H. diminuta, H. nana, Trichuris* spp., *C. hepaticum, Moniliformis moniliformis, Syphacia obvelata* and *Aspicularis tetraptera.* Kittipong *et al* (2010) found eggs of *H. nana, H. iminuta, Syphacia muris, C. hepaticum, Trichuris muris* and other strongyle eggs as well as adults of *S. muris* and segments of *H. nana* in the faeces of rats. The overall prevalence of helminth infections was higher in rats (100%) than that of mice (25.71%).

Lafferty *et al* (2010) recorded highest prevalence (12 nematodes per rat) of a stomach nematode, *Mastophorus muris* in *R. rattus* captured from coconut habitats. Milazzo *et al* (2010) also found that rats had a greater endoparasite prevalence rate (92.68%) than mice (61.64%). This could be related to rats' living (in sewage) and feeding (omnivorous) habits. All the 43 *R. rattus* samples were positive for parasites, while 25.71% of the 35 mouse samples were positive for one or more parasitic diseases. Rodrihuez-Vivas *et al* (2010) evaluated six species of rodents trapped from Yucata, Mexico for the presence of*C. fasciolaris* infection. Infection was prevalent only in two species i.e., *M. musculus* (9%) and *R. rattus* (3.5%). Prevalence was affected by sex and age of the host as males and adults were more infected than females and juveniles.

Gaherwal *et al* (2011) assessed the diversity of gastrointestinal parasites in *R. rattus* captured from Indore (India) and found a number of helminth species viz., *S. muris, Trichenella spiralis, T.muris, Ancylostoma duedenum, Heligomosomoides polygyrous, Nematospoiroideusdubius* and *A. tetraptera.* Goswami *et al* (2011) investigated 78 laboratory and wild rats in Izatnagar, India for parasitic diseases and found that 19.23% were infected with *H. diminuta.* Infection was higher in male rats than female rats. Gudissa *et al* (2011) found *H. diminuta*to be the most prevalent cestode in rats (44.18%) followed by *H. nana* (39.53%).

Jittapalapong *et al* (2011) in a parasitological survey detected *T. gondii* antibodies in 4.6% small rodents in Thailand using a Latex agglutination test. Kataranovski *et al* (2011) conducted a study on 302 rats of Serbia and found 68.5% rats infected with seven helminthic species viz. *Heterakis spumosum, Nippostrongylus brasiliensis, Calodium* spp., *T. muris, H. diminuta* and *Rodentolepis fraterna*. Out of these, *H. spumosa* and *H. diminuta* were most prevalent. Also, parasites were more prevalent in summer than in winter and spring seasons. Mehrabani *et al* (2011) found that rats from forested and urbanised regions had a higher prevalence of endoparasites showing a greater risk of infection to people.

Azizi *et al* (2012) examined blood smears of rodents trapped from the Jask district of Iran and found that females of various rodent species including *T. indica* were naturally infected by *Leishmania major*. Chaisiri *et al* (2012) investigated 725 rats trapped from various habitats of Thailand and concluded that overall prevalence rate of helminthes was 57.7%. Rats inhabited 14 nematodes, 3 cestodes, 3 trematodes and 1 acathocephalan with predominance of *Trichostrongyloid* spp. (24.3%) followed by *Raillientina* spp. (17.1%), *H. diminuta* (8.6%) and *S. muris* (8.6%). Herbreteau *et al* (2012) found that rodents collected from non-flooded lands, forests and paddy fields were comparatively more infested with pathogens.

Mohd Zain *et al* (2012) assessed parasitic load among rats (*R. rattus* and *R. Norvegicus*) in Malaysia and detected eleven species of helminth parasites including seven

nematodes (*H. spumosum, N. brasiliensis, S. muris, Pterygo dermatitis, Gongylonema neoplasticum* and *Angiostrongylus malaysiensis*), three cestodes (*H. nana, H. diminuta* and *T. taeniaeformis*) and one acanthocephalan (*M. moniliformis*). Overall, 80% of the rats carried at least one helminth species, with the highest prevalence being shown by *H. diminuta* (35%), *H. spumosum* (29.8%) and *H. nana* (28.4%). Mugisha *et al* (2012) for the first time reported the *Angiostrongylus cantonensis* infection in rats (6% of 33 observed) of Budongo Forest Reserve, Uganda. Sharma *et al* (2012) examined faeces of 43 *R. rattus* and 35 *M. musculus* collected from domestic areas and agricultural fields in tarai region of Uttarakhand (India) and found eggs of *H. nana, H. diminuta, S. muris, C. hepaticum, T. muris* and one unidentified strongyle worm in addition to adult worm of *H. nana* and *S. muris.*Siti *et al* (2012) examined 137 faecal and blood samples of rodents from urban areas of Kuala Lumpur, Malaysia and detected intestinal helminth parasites viz., *N. brasiliensis* (80.3%), *C. hepaticum* (13.9%), *H. nana* (23.4%) and *H. diminuta* (2.9%). Intestinal protozoan parasite recorded was *Entamoeba histolytica/Entamoeba dispar* (8.8%). *T. lewisi* (1.5%) was the only parasite detected from the blood samples.

Lee *et al* (2013) examined 46 wild rodents trapped from three different locations of Korea and found two species of trematodes (*Echinostoma hortense* and*Plagiorchis muris*), two species of cestodes (*H. nana* and *H. diminuta*) and three species of nematodes (*Ascaris* spp., *Syphacia* spp. and hookworms). Pakdel *et al* (2013) found 42.02% of the total rodents (138) from Kermanshah County, Iran infected with eight species of helminths (*S. muris, C. hepaticum, C. fasciolaris, A. tetraptera, H. spumosum, H. diminuta, S. obvelata* and *T. muris*). Scandola *et al* (2013) examined 94 non-commensal rodents (65 *Apodemus* spp. and 29 *Myodes glareolus*) trapped from forest area of Dijon, France to record the prevalence of hepatic infection. 27.6% *M. glareolus* and 1.5% *Apodemus* spp. were infected with *C. hepaticum* with no difference in prevalence with respect to sex and age of the host. Singla *et al* (2013) examined livers of *B. bengalensis* (18) for infection of *C. fasciolaris* and *C. hepaticum*. 44.4% rats experienced liver infection out of which 11.1% and 22.2% were infected with *C. hepaticum* and *C. fasciolaris*, respectively and 11.1% showed concurrent infection of both the species.

Bjelic-CaBrilo *et al* (2014) examined 44 small rodents in Zasavica, Serbia out of which 45% were found infected with different helminth parasitic species like *Calodium* spp., *Hymenolepis* spp., *Heterakis* spp., *Heligmosomum* spp., *Syphacia* spp., *Rictularia* spp., *Skrjabinotaenia* spp., *Trichocephalus* spp., *Rodentolepis* spp., *Brachylaima* spp. and *Plagiorchis* spp. Fuehrer (2014) reviewed distribution of *C. hepaticum* and concluded that *C. hepaticum* infection in muridae family was distributed among 60 countries of the world and more than 90 muridean species were infected with *R. norvegicus* being the predominant host globally. Garedhagi *et al* (2014) examined 36 *R. norvegicus*, 11 *R. rattus*, eight *M. musculus*

and two unknown species for endoparasitic diversity. No blood parasite was observed. *R. norvegicus* was found to inhabit five helminth species viz. *Trichosomoides crassicauda* (51.2%), *H. diminuta* (22.3%), *Gongylonema pulchrum* (12.1%), *H. nana* (4.31%) and *Trichocephal* spp. (2.18). *R. rattus* was infected with *T. crassicauda* (28.24%) and *G. pulchrum* (21.17%). Other species were free from parasites. McInnes *et al* (2014) reported the presence of *C. fasciolaris* cysts on the liver of laboratory rat, *R. norvegicus* in Australia.

Nateghpour *et al* (2014) examined 100 rodents (47 *T. indica*, 44 *M. hurrianae*, five *G. nanus* and four *M. libycus*) and found them harbouring endoparasites of zoonotic diseases (*H. diminuta, H. nana, Trichuris trichura, Skerjabino taenia, Ehrlichia muris* and *Leishmania* spp.). Noikong *et al* (2014) did molecular identification of identified various metacercariae of family Echinostomatidae collected from snails and rats using internal transcribed spacer 2 (ITS2) region of r-RNA and NADH-ubiquinone oxidoreductase chain 1 (ND1) region of mitochondrial DNA. The species identified were *Echinostoma revolutum, Echinostoma malayanum, Echinostoma robustum, Echinostoma trivolvis* and *Eupartphium albuferensis*. Ogunniyi *et al* (2014) reported 58% and 96% of the rats collected from of Nigeria inhabited with five genera of helminths and seven genera of protozoans, respectively. Priyanto *et al* (2014) examined 147 rats from houses, gardens, rice fields and traditional markets of Java and recovered *C. hepaticum* and *T. taeniaeformis* from liver, *S. muris* from caecum, *Mastophorus* spp. and *G. neoplasticum* from stomach and *H. nana, H. diminuta*, *N. brasiliensis*, *M. moniliformis* and *Echinostoma* spp. from intestine. Sinniah *et al* (2014) found the liver of 64.3% urban rats (*R. rattus* and *R. norvegicus*) infected with *C. hepaticum* (44.9%) and *C. fasciolaris* (39.3%). Liver of 20.4% rats were infected with both the parasites.

Aghazadeh *et al* (2015) reported 16.5% prevalence of *Angiostrongylus* spp.in liver and faeces of its definitive host (*Rattus* spp.) in the form of adults and larvae. Chaisiri *et al* (2015) examined 2478 rodents trapped from four different habitats (human settlements, irrigated land, forests and non-flooded lands) of Thailand, Cambodia and Lao PDR and recovered *Cyclodontostomum purivisi*, *Echinostoma ilocanum*, *E. malayanum, H. diminuta*, *H. nana, M. moniliformis*, *P. muris* and *Raillientina* spp. in 29.7% of the rats. Singh and Arya (2015) reported a natural co-infection of *C. fasciolaris* and *H. diminuta* in 25 male Wistar rats which might have resulted in their sudden unexplained mortality.

Azzam *et al* (2016) examined eight species of rodents collected from Egypt and found them infected with three nematode species (*A. cantonensis, Trichris* sp. and *Enterobius vermicularis*) and one cestode species (*H. nana*). Infection rate was 56.67% in *R. rattus frugivorous*, 26.67% in *R. rattus alexandrines* and 50% in *R. norvegicus*. Gholipoury *et al* (2016) conducted a parasitological survey on 91 wild rats of Turkmen Sahra and found 38.5% infection of *H. diminuta* (7.7%), *Cryptosporidium* spp. (6.6%), *Trichuris* spp. (5.5%), *C. fasciolaris* (2.20%), *Angiostrongylus* spp. (2.20%), *Calodium* spp. (1.09%), *Rhipicephalus*

spp. (8.70%), *N. fasciatus* (1.09%) and *L. nuttalli* (3.29%). All the parasites except *L. Nuttalli* and *N. fasciatus* were of zoonotic importance. Moudgil *et al* (2016) examined 45 Wistar rats for histopathology and morphology of *C. fasciolaris*in liver. 17.78% rats had the infected liver showing inflammation, metaplasis, tissue granulation and fatty changes. Premalatha *et al* (2016) trapped 105 rats of *Rattus* spp. from Kaula Lumpur, Malaysia and found cysts of *C. fasciolaris* in nine rats.

Rahdar *et al* (2016) studied 30 rodents including *M. musculus*, *R. norvegicus* and *T. indica* captured from different parts of Ahvaz southwest of Iran and identified three species of cestodes, eight species of nematodes, one species of acanthocephalans and three genera of protozoan parasites with predominance of *H. nana* and *Strongyloides ratti*. Seifollahi *et al* (2016) examined stool samples of rodents trapped from Boyer-Ahmad district, Iran and found protozoa infection in 53.8% cases. Antibodies to *T. gondii* were detected in the sera of 9.6% cases. Singla *et al* (2016) screened 83 mature *B. bengalensis* trapped from premises near railway station, fish market and agricultural fields and reported the occurrence of *H. diminuta, H. nana, C. fasciolaris* and *C. hepaticum* in 68.67% of the rats. Songsri *et al* (2016) reported the effect of natural infection of *E. malayanum* on intestinal histology and growth of hamsters, rats, mice and gerbils. Tresnani *et al* (2016) examined 30 *R. rattus* for endoparasitic fauna and observed six species of helminths (*H. nana, H. diminuta, Strongyloides* sp., *Trichostrongylus* sp., *Trichuris* sp. and *Moniliformis dubius*).

Archer *et al* (2017) examined 400 wild rodent species and found them infected with *Gongylonema* spp. (25.3%), *H. diminuta* (17.2%), *T. lewisi* (22.8%), *A. cantonensis* (15.3%), *T. gondii* (11.2%), *M. moniliformis* (9.5%), *C. hepaticum* (2.6%) and *H. nana* (0.8%). Arzamani *et al* (2017) dissected 113 rats and recorded the presence of helminths such as *A. tetraptera*, *H. diminuta*, *N. brasiliensis*, *Protospirura seurat*, *Rictolaria ratti*, *Skrjabinitaenia lobata*, *Streptophara guskuntzi*, *S. oblevata*, *T. taeniaeformis*, *T. muris*, *C. fasciolaris*, *Acanthocephal* spp. and *Trichuris* spp. Jarvi *et al* (2017) euthanized 545 rats (*R. rattus* and *R. exulans*) trapped from multiple sites of Hawaiian Island and were examined for lung parasites. 93.1% rats were infected with *A. cantonensis*.

Mendenhall *et al* (2017) for the first time reported *Angiostrongylus* species infecting rodents in Singapore. They also detected the presence of medically important gastrointestinal helminths in rodents viz. *Dicrocoelium dendriticum* and *M. moniliformis*. Ranjbar *et al* (2017) examined 52 rodents of different species for helminthic diversity and reported an overall infection rate of 73%. Helminthes recovered were *H. diminuta* (50%), *T. muris* (36.5%), *H. nana* (28.8%), *A. tetraptera* (15.4%), *Anoplociphalidae* spp. (15.4%), *Skrjabinotaenia* spp. (15.4%), *Rictularia* spp. (15.4%), *C. fasciolaris* (5.8%), *Syphacia* spp. (5.7%), *Gongylonema* spp. (3.8%) and *Trichostrongylus* spp. (3.8%). Sharma *et al* (2017) observed liver of 115 out of 170 *R. norvegicus* trapped from various locations of West Indies infected with *C.*

fasciolaris. Prevalence was observed to be affected by sex and age of the host. Females were more affected (74.7%) than males (60.9%). Similarly, adults experienced more infection (70.7%) than young ones (45%). Yang *et al* (2017) conducted a study in China on diversity of *H. nana* (6.1%) and *H. diminuta* (14.9%) in *R. norvegicus*. Molecular characterisation was done by amplifying mitochondrial cytochrome c oxidase subunit 1 (COX 1) gene and ITS2 region of nuclear ribosomal RNA gene.

Fantozzi *et al* (2018) for the first-time reported *C. hepaticum* infection in Sigmodontinae rodents from Argentina. Molecular characterisation of 18S ribosomal RNA gene region of the parasite was also done. Moradpour *et al* (2018) examined respiratory and gastrointestinal tracts of 253 rodents belonging to five species viz. *T. indica*, *M. libycus*, *M. musculus*, *Apodemus witherbji* and *Calomyscus elburzensis* trapped from Iran. They concluded that 20.1% rats were infected with *S. oblevata*, 9.9% with *A. tetraptera*, 6.7% with *N. brasiliensis*, 4.3% with *Heligmosomoides polygyrus*, 3.1% with *H. diminuta*, 2.7% with *C. fasciolaris*, 1.1% with *Gongylonema* spp., 0.8% with *H. nana*, 0.7% with *Physaloptera* spp. while, *T. muris*, *Calodium* spp., *M. moniliformis* and *Mesocestoides* spp. infected only 0.3% of the rodents. Only species of trematodes found infecting rodents was *Notocotylus neyrai*.

Rabiee *et al* (2018) reported plague, leishmaniasis and hymenolepiasisas the most frequent diseases found among commensal rodent (*R. norvegicus*, *M. musculus* and *R. rattus*) populations in Iran. Rael *et al* (2018) examined lungs of rodents captured from various sites of New Orleans, Louisiana, USA and detected presence of *A. cantonensis* with overall prevalence rate of 38%. Sharma *et al* (2018) investigated faeces of 78 rodents collected from Tarai region of Uttarakhand, India and found 100% *R. rattus* (43) and 25% *M. musculus* (total 35) infected with helminths i.e., *H. nana, H. diminuta, S. muris, C. hepaticum* examined faecal samples of 586 rodents from pet shops and breeding clubs of Slovakia for prevalence of oxyurid nematodes. Four species of nematodes identified in order of prevalence were *S. muris, S. obvelata, A. tetraptera* and *Paraspido derauncinata*.

Begum *et al* (2019) examined 50 *R. rattus* collected domestic area and paddy field area of Dhaka, Bangladesh and reported 88% prevalence of helminth parasites having intensity of 34.73 (1528 parasites from 44 hosts). Intestine and liver of these rats were infected with one trematode, four cestode and three nematode species. *Echinostoma cinetorchis* (36.52%) was the predominant one followed by *Heterakis spumosa* (31.74%), *H. nana* (7.26%), *S. obvelata* (7.13%), *Hydatigera taeniaeformis* (6.68%), *Oesophagostomum eurycephalum* (5.04%), *H. diminuta* (3.01%) and *Hymenolepis* sp. (2.62%). Domestic rats showed the highest prevalence of infestation (90%) compared to paddy field rats (85%), whereas the intensity was lower in domestic rats than in the paddy field rats. Higher prevalence was observed in male rats but intensity was higher in female rats.

Spickett *et al* (2019) trapped 1030 rodents from various sites of South Africa and

examined the helminthic diversity in them. They recovered 15 nematode species (*Abbreviata*, *Ascarops*, *Aspicularis*, *Heligomonina*, *Mastophorous*, *Maupasina*, *Nematodirus*, *Neoheligmonella*, *Paralibyostrongylus*, *Protospirura*, *Streptopharagus*, *Subulura*, *Syphacia*, *Trichostrongylus* and *Trichuris*), nine cestode species (*Afrobaeria*, *Hymenolepis*, *Inermicapsifer*, *Mathenotaenia*, *Meggittina*, *Raillientina*, *Rodentolepis*, *Skrjabinotaenia* and *Sudarikovina*) and one species of acanthocephalan (*Moniliformis* sp.) under different genera of helminth parasites.

Tijjani *et al* (2020) recorded endoparasitic diversity in *R. rattus* and *R. norvegicus* at Selangor, Malaysia. Out of 89 rodents, 15.4% and 17.1% were found infected with at least one species of parasites, respectively. Total twelve parasitic species were reported with prevalence rate i.e., *T. taeniaeformis* (28%), *H. nana* (19.5%), *H. diminuta* (16.1%), *C. hepaticum* (19.1%), *Trichuris* spp. (12.3%), *Cryptosporidium* spp. (21.3%), *E. histolytica* and *M. moniliformis* (17.9%), *A. cantonensis* (16.8%), *Giardia* sp. (14.6%), *T. gondii* (6.7%), and *Sarcocystis* spp. (6.74%).

Bimi *et al* (2021) observed 61.8% of the total rodents infected with one or more of the parasites (*Toxascaris* sp., *Isospora* sp., *Hymenolepis* sp., *Trichuris* sp., *Ascaris* sp. and *Taenia* sp.). Out of these, 17.6% had single parasite infections, while 44.1% had multiple infections. Brar *et al* (2021a) gave a comprehensive report on patho-physiological alterations along with morpho-molecular characterization and risk assessment of natural infections of *H. diminuta* and *H. nana* in 291 commensal rodents (*R. rattus* and *B. bangalensis*). Small intestine of 53.61% and 64.95% rats was found infected with *H. diminuta* and *H. nana*, respectively with a concurrent infection rate of 50.86%. The host age had significant effect on the prevalence of two parasites. Brar *et al* (2021b) did molecular characterization of two helminthic species (*T. taeniaeformis* and *C. hepaticum)* found in commensal rodents in Punjab (India). These endoparasites were present either alone (4.33-6.33%) or as mixed infection (65.55%). The level of total proteins and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were found significantly higher in the liver of rodent species infected with single and mixed infection compared to those with no infection.

Galán-Puchades *et al* (2021) analysed urban population of *R. norvegicus* in the city of Barcelona, Spain for the presence of zoonotic intestinal protozoans. In total 100 rats trapped, four protozoan species were identified viz. *Blastocystis* sp. (83.5%), *Giardia duodenalis* (37.7%), *Cryptosporidium* spp. (34.1%) and *Dientamoeba fragilis* (14.1%). Mandla *et al* (2021) analysed 180 *T. indica* trapped from crop fields of Punjab (India) for the prevalence of nematode parasites in liver and gastrointestinal tract. Only 18.33% gerbils were found infected with four nematodes species *i.e., N. brasiliensis* (39.40%), *C. hepaticum* (21.21%), *T. muris* (21.21%) and *S. muris* (18.18%). Eggs of *N. brasiliensis*, *T. muris* and *S. muris* were found in faecal samples and pale-yellow lesions of *C. hepaticum* were found on surface of

liver.

2.3 Case reports in humans

Rodent borne zoonotic diseases are threat to public health and have emerged as epidemic from time to time. Besides causing mortality in humans and animals, the parasites can complicate the health status by inducing alterations in physiological and immunological mechanisms of the host resulting into tissue damages, stimulating abnormal tissue growth, competing with the host for nutrients, decreasing the volume of host's blood and body fluids and by mechanical interference (Hsu 1980, Aboel-Hadid and Allam 2007).

In wild rodents, the eggs of *C. hepaticum* may be liberated from the liver into the external environment (soil/water) by the natural death of the host and decomposition of the body, from where it can infect humans and other animals (Spratt and Singleton 2001). Although rare, hepatic capillarasis caused by *C. hepaticum*, is wide spread in humans from different parts of the world. It infected more than 1300 persons in 1967-68 and 1978-79 and over 90 persons have died in Philippine due to this disease (Bair *et al* 2004, Fuehrer 2014). Rocha *et al* (2015) recorded that, out of 490 residents in urban area of Porto Velho, Brazil, 1.8% population was infected with *C. hepaticum.* Another example is a case report of a severe infection in a 5-year-old child of Iran (Aghdam *et al* 2015).

Strongyloidiasis infects millions of people worldwide and is an important cause of mortality from intestinal helminth parasitic infection in developed countries. Boulware *et al* (2007) found 151 individuals infected with strongyloidiasis. Out of 1291 positive stool specimens collected, *Strongyloides stercoralis* was the third most common helminth parasite identified accounting for 11% of potentially pathogenic gastrointestinal parasites

Hymenolepiasis caused by both the cestodes species (*H. nana* and *H. diminuta*) having wide range of prevalence values, is more common in areas of poor structural and socio-environmental conditions and where there is close contact between rodents and humans. Although young children can be infected with *H. nana* eggs from rodent sources, this type of infection is probably less common in them (Faust *et al* 1962). Dispersal of the eggs of *H. diminuta* in the environment via beetle faeces (Pappas and Barley 1999, Zhong *et al* 2013, Makki *et al* 2017) represents an additional source of infection. In the unusual life cycle of *H. nana*, where no intermediate host is essential, man is probably at the main risk of infection. *H. nana* is the most common human dwarf tapeworm. Its prevalence among children is as high as 25% (Crompton 1999). About 9.9% prevalence of *H. nana* was found among urban slum dwellers in India (Mirdha and Samantary 2002). Hymenolepiasis due to *H. nana* has been reported to affect up to 75 million persons worldwide (Peters and Pasvol 2002). Both *H. diminuta* and *H. nana* have been reported in humans particularly the children (Alvez *et al* 2003).

A case of *H. diminuta* infection was recorded by Tena *et al* (1998) in a 5-year-old

child from Guadalajara, Spain. *H. diminuta* eggs were detected in faecal samples. Since the initial random studies on 10,000 human stool samples revealing 0.23% samples positive for eggs of *H. diminuta* in India (Chandler *et al* 1923), sporadic cases of hymenolepiasis have been frequently reported from India (Watwe and Dardi 2008) and other parts of the globe (Marangi *et al* 2003). Another case report of *H. diminuta* infection was reported in 2-year-old child living in a suburban area of Catania, Italy (Patamia *et al* 2010). Alvarez-Fernández *et al* (2012) presented a case of mixed infection of *H. nana* and *H. diminuta* in a mother and her 12-year-old child in an urban area of Chilpancingo, México. Karuna and Khadanga (2013) reported a case of *H. diminuta* infection in an 18-year-old male farmer having symptoms like pruritic maculopapular rashes and dull aching left iliac fossa pain for 4-6 months. Kolodziej *et al* (2014) presented a case of 3-year-old boy infected with *H. diminuta* in Poland. Pérez-Chacón *et al* (2017) reported a case of co-infection with *H. nana, H. diminuta, Giardia intestinalis* and Human Immunodeficiency Virus (HIV) in a 43 year old man from Venezuela. Kandi *et al* (2019) described a case of *H. nana* infection in a 24-year-old pregnant woman who was in her 35th week of pregnancy. Direct stool examination indicated a high infestation of eggs/ova that morphologically resembled *H. nana* and was used to make the diagnosis.

In a case report presented by Andre *et al* (2005), a 7-year-old child owing pet rats developed an eruptive fever with blisters, polyarthritis, and spectacular desquamation of the hands. An acanthocephalan parasite, *Streptobacillus moniliformis* was identified after culture of the child's blister fluid and was also detected in rat samples by molecular diagnosis techniques. After five days of antibiotic treatment, the patient's condition was improved. Berenji *et al* (2007) reported a case of *M. moniliformis* infection in 2-year-old girl in Iran. Previously two cases of *M. moniliformis* infection were reported in Iran and this was the third case. Another case report of *M. moniliformis* infection in humans was given by Salehabadi *et al* (2008) in Iran. A female worm was obtained from a child, but the source of infection to the child was unknown.

Echinostoma is the largest genus of trematodes with many species known to infect humans (Sohn and Na 2017). Echinostomiasis is a food-borne parasitic disease (Toledo *et al* 2014) reported from all the continents (Poland *et al* 1985). Human echinostomiasis is an emerging yet neglected public health disease attributed to at least 24 echinostome species endemic to Southeast Asia and the Far East with major foci located in China, India, Indonesia, South Korea, Malaysia, the Philippines and Thailand (Toledo and Esteban 2016). Human infections with regard to *Echinostoma* spp. seem to be relatively rare (Chai 2009). The first human infection with *E. malayanum* was reported by Leiper (1911) in labourers of Indian origin in Malaysia. Maji *et al* (1993) reported a case of two tribal men infected with *E. malayanum* near Calcutta, India. Three patients from Philippines were reported to have operculated eggs and adult flukes recovered from stool samples which were identified as *E.*

malayanum (Belizario *et al* 2007). All the patients had the history of consuming raw fish dipped in salt and vinegar mixture. Chai *et al* (2012) reported echinostomiasis as one of the endemic trematode infections among the residents of Khammouane Province, Lao PDR.

In India, *A. sufrartyfex* infection was reported for the first time from a girl in Assam who died of infection (Lane 1915). From 2004-2017, 170 cases of *Artyfechinostomum sufrartyfex* infection were recorded in children of Bihar, India during which 11 children died (Prasad *et al* 2019). In most of the cases infection had occurred due to consumption of raw snails. Sah *et al* (2018) reported a case of echinostomiasis in a 62-year-old man from Nepal. He had history of consuming insufficiently cooked fish and snail with alcohol. During endoscopy, an adult flat worm identified as *Echinostoma* species was found in stool sample. Borkataki *et al* (2017) found a dead Indian mongoose in Jammu and Kashmir, India which was infected with *A. sufrartyfex*.

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. 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). Giardiasis 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). Concurrent infection of *Cryptosporidium* and *Giardia* has been reported in calves on two Ohio farms and humans in Africa (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 ectoparasite, *X. cheopis* reported in different studies is the most important vector of plague and the rickettsial infection of murine typhus (Fagir and El-Rayah 2009). The cestode parasite, *H. diminuta* can also be transmitted to humans by this flea species (VirojWiwanitkit 2004). The blood sucking lice such as *Hoplopleura* spp. cause pruritus, alopecia, dermal irritation and even anemia (Dong *et al* 2014). The spiny rat mite, *Echinolaelaps echidninus* also parasitizes man and cause dermatitis. A number of tick species act as vectors of lethal pathogens (protozoa, bacteria, rickettsiaand arboviruses) and are ranked second after mosquitoes (Ghosh and Nagar 2014). The important tick-borne rickettsial diseases in India are Q fever and Indian tick typhus (Geevarghese and Mishra 2011). Tick bites also cause stress and blood loss to the animal and human hosts (Rahdar *et al* 2015).

Thus, the studies on risk assessment of rodent-borne diseases in relation to public health have shown that rodents can spread pathogens to humans either directly through biting, breathing in germs and contaminating food and water or indirectly via ectoparasitic arthropod vectors.

CHAPTER III

MATERIALS AND METHODS

The lesser bandicoot rat, *B. bengalensis* examined in the present study for the presence of ecto and endoparasites of zoonotic importance were collected from fish market and railway station at Ludhiana, Punjab, India from November, 2020 to October, 2021. The whole period was divided into three seasons i.e., winter (November-February), summer (March-June) and monsoon (July-October).

3.1 Collection and maintenance of animals

Total 100 *B. bengalensis* of both sexes were live trapped from the above-mentioned study areas (50 from each location) using multi catch live traps. Traps were baited with pieces of Indian chapatti and placed near rodent burrows and other rodent activity sites in the evening hours. Rats caught in the next morning were taken to the laboratory and after noting their sex, weight and maturity status, they were kept in individual cages. The existence of scrotum and vaginal orifice were used to differentiate male and female rats. Rats of body weight <150g were categorized as young while those ≥150g were considered as adults. Food and water were provided *ad libitum*. Food consisted of mixture of broken wheat, edible vegetable oil and powdered sugar in ratio 96:2:2. Animals were maintained in the laboratory as per the guidelines of Institutional Animal Ethics Committee.

3.2 Examination for ectoparasitic infestation

During examination for ectoparasites, the rats were given mild anaesthesia and placed on a white tray. Their fur, limbs, axillary region, snout and ears were combed using a fine-toothed comb to dislodge the ectoparasites if any in the white tray. The ectoparasites fallen in the tray were counted and collected using a fine hair brushing labelled vials and preserved in 70% ethyl alcohol for further identification. For identification the parasites were mounted on the slide and observed under a light microscope. The parasites were identified using identification key given by Taylor *et al* (2016).

3.3 Examination for endoparasitic infection

3.3.1 Faecal examination

A plastic tray was kept under the cage of each of the trapped *B. bengalensis* to collect faeces samples. The presence of parasite eggs was detected by processing 10 faecal droppings from each animal**.** Following methods were used to detect the presence of parasitic eggs in the faecal samples:

3.3.1.1 Method of flotation

Principle: It works on the principle that if parasitic eggs are suspended in a liquid with a specific gravity higher than eggs, the latter will float to the surface and be collected for microscopic examination. In the flotation process, saturated sodium chloride solution is

typically utilised. It is affordable, simple to make and efficient against floating parasite eggs. Nematode and cestode eggs float in a liquid with a specific gravity of 1.18-1.20 (Taylor *et al* 2016).

Procedure

1. Took 10 ml saturated salt solution in a pestle mortar and triturated 10 fresh faecal pellets in it.
2. The mixture was poured into a glass beaker through a tea strainer.
3. Took a small glass vial and filled it with the above fluid up to the rim.
4. Then put a cover slip over the rim of the vial.
5. Kept the vial for about 15-20 minutes for the floatation to settle. Vial was not allowed to stand for too long to avoid crystallization of salt on the edge of cover slip.
6. Removed the cover slip from the vial and kept on a slide.
7. The slide was observed under the microscope for the presence of eggs firstly at 100x and 400x magnification.

3.3.1.2 Method of sedimentation

Principle: It works on the principle that when parasite eggs are suspended in a liquid with a specific gravity lower than that of eggs, the latter will sink to the bottom. The technique can be used to investigate much heavier eggs with a specific gravity of 1.30-1.35.

Procedure

1. Took 10 ml tap water in a pestle mortar and triturated 10 fresh faecal pellets in it.
2. The mixture was poured into a glass beaker through a tea strainer.
3. Took a small glass vial and filled it with the above fluid.
4. Kept the vial for 15-20 minutes after which discarded the upper half of the fluid.
5. With help of a dropper, placed a drop of the sediment on a glass slide and covered it with a cover slip.
6. Examined the slide under the microscope for the presence of eggs at 100x and 400x magnification.

3.3.2 Micrometry of eggs

The ocularmicrometer was used to measure the dimensions of eggs of different endoparasites at 100x and 400x magnifications which was then calibrated with stage micrometer. The length and breadth of eggs of different endoparasites were measured using the method given by Soulsby (1982). Eggs of different kinds found in present study were also micro photographed.

3.3.3 Examination of visceral organs

The rats were dissected after sacrificing with chloroform. A mid-ventral incision was used to expose the visceral organs, which were then examined macroscopically for the presence of endoparasites. Different organs examined were liver, stomach, small intestine and

large intestine. These organs were dissected out and transferred into the petri dishes containing normal saline solution. In the petri dish, the organs were cut open to retrieve the adult worms. The adult worms seen through the naked eye were counted and collected and preserved in a labelled vial containing 70% ethyl alcohol for further identification. The parasites were micro photographed and identified using identification key given by Taylor *et al* (2016). The following parameters were calculated using the formulae given below and described in Bush *et al* (1997) based on the data obtained:

$$\text{Per cent hosts infected} = \frac{\text{Number of hosts infected}}{\text{Number of hosts examined}} \times 100$$

$$\text{Per cent parasite prevalence} = \frac{\text{Number of parasites}}{\text{Total number of parasites}} \times 100$$

$$\text{Parasite intensity} = \frac{\text{Number of parasites}}{\text{Number of hosts infected}}$$

$$\text{Parasite index} = \frac{\text{Number of parasites}}{\text{Number of hosts examined}}$$

3.3.4 Molecular characterization of *N. brasiliensis*

Molecular characterization of *N. brasiliensis* adults found in small intestine was conducted to confirm their identity. Genomic DNA of the adult parasites preserved in 70% ethyl alcohol was extracted using QIAamp tissue kit (Qiagen, Hilden, Germany) as per the manufacturer's protocol with slight modifications. The parasites were mechanically disrupted by using sterile pestle-mortar. Final elutions of DNA were made in 20-100 µl of elution buffer. The genomic ribosomal DNA extracted from the parasites was used in PCR to amplify the mitochondrial cytochrome oxidase I (COX1) gene (355 bp). The forward (5′-GTTTGGTTTGTGGTCGGGT-3′) and reverse (5′-CCGGGATGACCCAAAGTTCT-3′) primers were self-designed from *N. brasiliensis* (GenBank accession no. AP017690) using Primer-BLAST. PCR was carried out in the thermo cycler in a total volume of 25 µl comprising of 12.5µl of master mix, 4.5µl of nuclease free water, 1.5µl of forward and reverse primers each and 5µl of extracted DNA. Reaction conditions for PCR consisted of an initial denaturation at 95ºC for 1 min followed by 30 cycles of denaturation at 94ºC for 45 s, annealing at 54 ºC for 1 min, extension at 72ºC for 1 min and a final extension at 72ºC for 5 min. During each amplification reaction, a template control was also included in each plate as negative control.

Amplification products were analyzed on 1.5% agarose gel and visualized by ethidium bromide staining. PCR products were purified using QIAquick@ PCR purification

kit as per the manufacturer's protocol. The identity of PCR product was confirmed after sequencing from Bioserve Biotechnologies, Hyderabad, Telangana, India and putting sequences obtained to Basic Local Alignment Search Tool (BLAST 2.2.22). Then the sequences were aligned using ClustalW multiple alignment tool with the default gap. The phylogenetic tree was constructed by comparing the COI gene of the *N. brasiliensis* with other sequences from GenBank using maximum likelihood method and Hasegawa-Kishino-Yano model in MEGA X (Hasegawa *et al* 1985, Kumar *et al* 2018). The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analysed. Evolutionary analyses were conducted in MEGA X (Kumar *et al* 2018).

3.4 Statistical analysis

The prevalence of different ecto and endoparasites was subjected to risk factor analysis in relation to different seasons, host age and sex using Pearson's chi-square test at 5% level of significance. Logistic analysis was conducted using SAS software version 9.3. The relative risk was determined as per the method described in Thrusfield (2005). Confidence interval for relative risk was calculated by the formula given below:

$$= RR^{1 \pm 1.96/\chi}$$

Where RR = relative risk, and

$$\chi = \sqrt{\text{Chi}^2 \text{ value}}$$

CHAPTER IV

RESULTS AND DISCUSSION

The present study was conducted on distribution of *B. bengalensis* at two different locations and ecto and endoparasites infecting them in relation to various epidemiological factors such as season, location, host sex and age, along with the relative risk of zoonotic disease transmission. The results are presented herewith.

4.1 Distribution pattern of rats collected

4.1.1 Distribution of rats collected from fish market

Total 50 rats comprising 33 females and 17 males were collected from Fish market, Ludhiana from November, 2020 to October, 2021. The collection was made in all the three seasons. Rats collected in winter, summer and monsoon seasons were 27, 11 and 12, respectively. Number of female rats collected was more than male rats in all the three seasons (Table 1). In total 50 animals, 30 were young and 20 were adult rats. The number of young individuals collected was more in winter (17) and monsoon (9) seasons, while the number of adults collected was more in summer season (7) (Table 2).

The number of young females (17) collected was more than young males (13) and similarly, the number of adult females (16) collected was also more than adult males (4). In female rats collected, the number of young (17) and adult (16) individuals was almost the same while in male rats collected, the number of young (13) individuals was more than adult (4) individuals (Table 3).

Table 1. Sex wise distribution of *B. bengalensis* collected from fish market in different seasons

Sex	Winter	Summer	Monsoon	Overall
Female	15(55.56)	10(90.91)	8(66.67)	33(66.00)
Male	12(44.44)	1 (9.09)	4(33.33)	17(34.00)
Total	**27(100.00)**	**11(100.00)**	**12(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

Table 2. Age wise distribution of *B. bengalensis* collected from fish market in different seasons

Age	Winter	Summer	Monsoon	Overall
Young	17(62.96)	4(36.36)	9(75.00)	30(60.00)
Adult	10(37.03)	7(63.63)	3(25.00)	20(40.00)
Total	**27(100.00)**	**11(100.00)**	**12(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

Table 3. Sex and age wise distribution of *B. bengalensis* collected from fish market

Sex/Age	Young	Adult	Total
Female	17(56.66)	16(80.00)	33(66.00)
Male	13(43.33)	4(20.00)	17(34.00)
Total	**30(100.00)**	**20(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

Overall distribution of *B. bengalensis* collected from fish market season, sex and age wise is given in Table 4 and Figures 1-3. In winter season total 27 rats, comprising 15 female (9 young and 6 adult) and 12 male (8 young and 4 adult) rats were collected. In summer season 11 rats, comprising 10 females (3 young and 7 adult) and 1 young male rat were collected. In monsoon season, 12 rats comprising 8 females (5 young and 3 adult) and 4 young male rats were collected. No adult male rats were collected in summer and monsoon seasons. In overall, young individuals collected were more (17, 4 and 9) as compared to adult individuals (10, 7 and 3) in winter, summer and monsoon seasons, respectively.

Table 4. Season, sex and age wise distribution of *B. bengalensis* collected from fish market

Season/sex/age	Female		Male		Overall
	Young	Adult	Young	Adult	
Winter	9(52.94)	6(37.50)	8(61.53)	4(100.00)	27(54.00)
Summer	3(17.64)	7(43.75)	1(7.69)	-	11(22.00)
Monsoon	5(29.41)	3(18.75)	4(30.76)	-	12(24.00)
Total	**17(100.00)**	**16(100.00)**	**13(100.00)**	**4(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

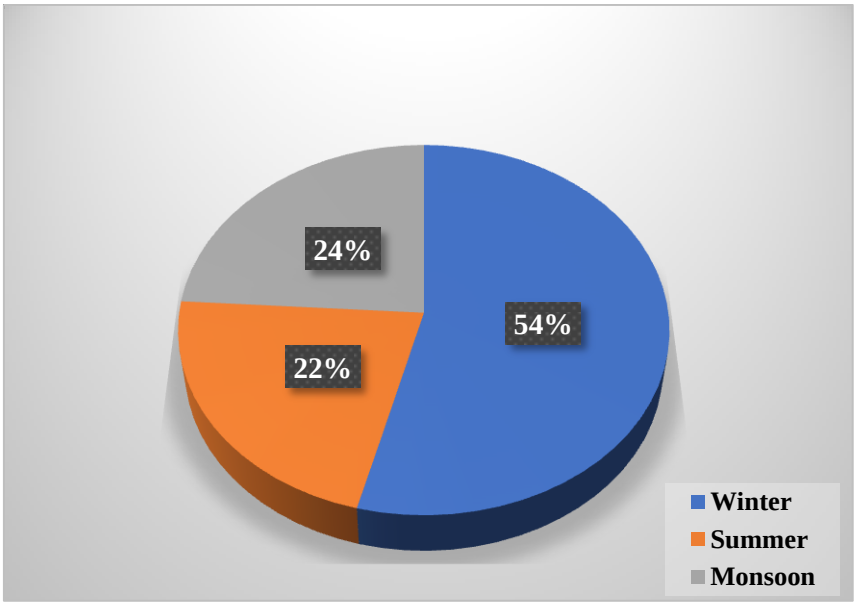

Figure 1. Season wise distribution of *B. bengalensis* collected from fish market

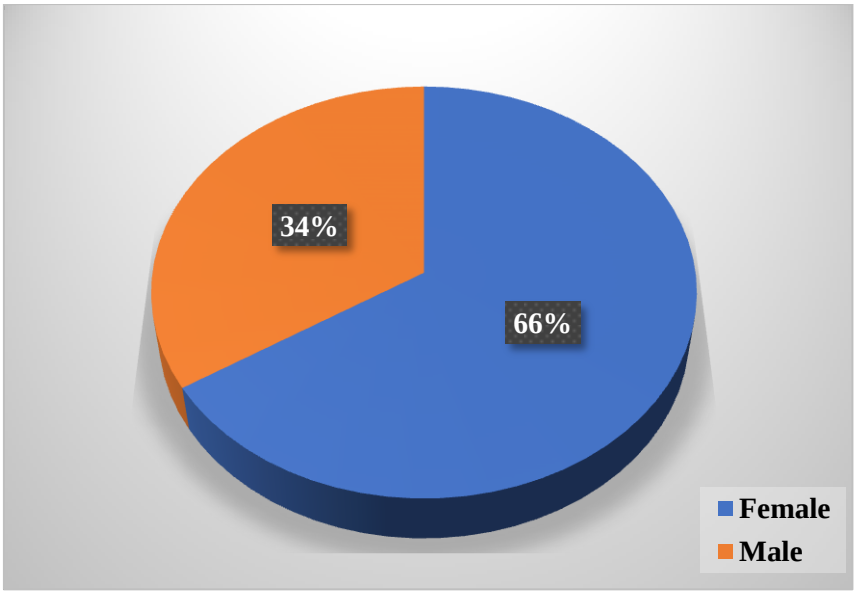

Figure 2. Sex wise distribution of *B. bengalensis* collected from fish market

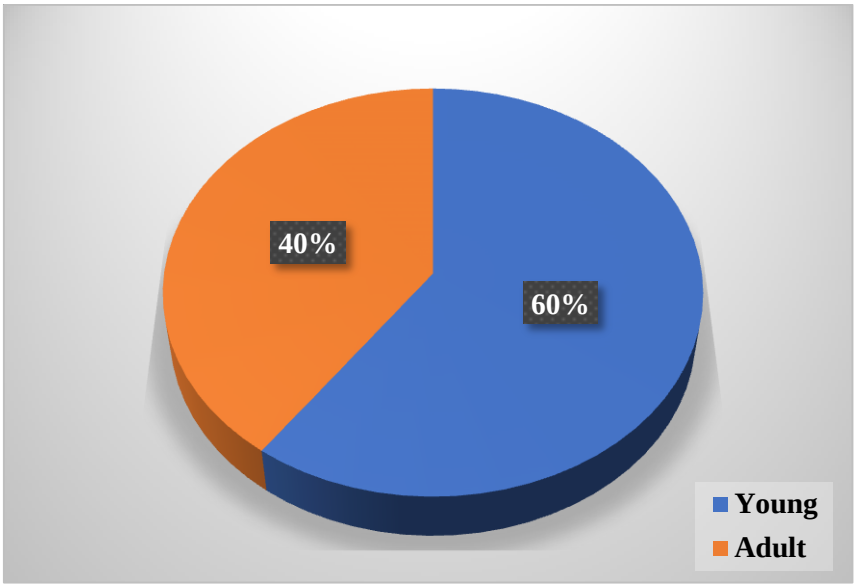

Figure 3. Age wise distribution of *B. bengalensis* collected from fish market

4.1.2 Distribution of rats collected from railway station

Total 50 rats comprising 27 females and 23 males were collected from railway station, Ludhiana from November, 2020 to October, 2021. The collection was made in all the three seasons. Rats collected in winter, summer and monsoon seasons were 12, 13 and 25, respectively (Table 5). In total 50 animals, 22 were young and 28 were adult rats. The number of young individuals collected was more in summer (10) season, while the number of adults collected was more in monsoon season (19). The number of young and adults collected was same in winter season (Table 6).

The number of young females and males collected was equal (11 each) while the number of adult females (16) collected was also more than adult males (12). In male rats collected, the number of young (11) and adult (12) individuals was almost the same while in

female rats collected, the number of young (11) individuals was less than adult (16) individuals (Table 7).

Table 5. Sex wise distribution of *B. bengalensis* collected from railway station in different seasons

Sex/season	Winter	Summer	Monsoon	Overall
Female	8(66.66)	7(53.84)	12(48.00)	27(54.00)
Male	4(33.33)	6(46.15)	13(52.00)	23(46.00)
Total	**12(100.00)**	**13(100.00)**	**25(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

Table 6. Age wise distribution of *B. bengalensis* collected from railway station in different seasons

Age/season	Winter	Summer	Monsoon	Overall
Young	6(50.00)	10(76.92)	6(24.00)	22(44.00)
Adult	6(50.00)	3(23.00)	19(76.00)	28(56.00)
Total	**12(100.00)**	**13(100.00)**	**25(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

Table 7. Age wise distribution of male and female *B. bengalensis* collected from railway station

Sex/age	Young	Adult	Overall
Female	11(50.00)	16(57.14)	27(54.00)
Male	11(50.00)	12(42.85)	23(46.00)
Total	**22(100.00)**	**28(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

Overall distribution of *B. bengalensis* collected from railway station season, sex and age wise is given in Table 8 and Figures 4-6. In winter season total 12 rats, comprising 8 female (3 young and 5 adult) and 4 male (3 young and 1 adult) rats were collected. In summer season 13 rats, comprising 7 females (6 young and 1 adult) and 6 male (4 young and 2 adult) rats were collected. In monsoon season, 25 rats comprising 12 females (2 young and 10 adult) and 13 male (4 young and 9 adult) rats were collected. Only one adult female and one adult male rat were collected in winter and summer seasons, respectively. In overall, adult individuals collected were more in monsoon season (19) and young individuals collected were more in summer season (10).

Table 8. Season, age and sex wise distribution of *B. bengalensis* collected from railway station

Season/sex/age	Female		Male		Overall
	Young	Adult	Young	Adult	
Winter	3(27.27)	5(31.25)	3(27.27)	1(8.33)	12(24.00)
Summer	6(54.54)	1(6.25)	4(36.36)	2(16.66)	13(26.00)
Monsoon	2(18.18)	10(62.50)	4(36.36)	9(75.00)	25(50.00)
Total	**11(100.00)**	**16(100.00)**	**11(100.00)**	**12(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

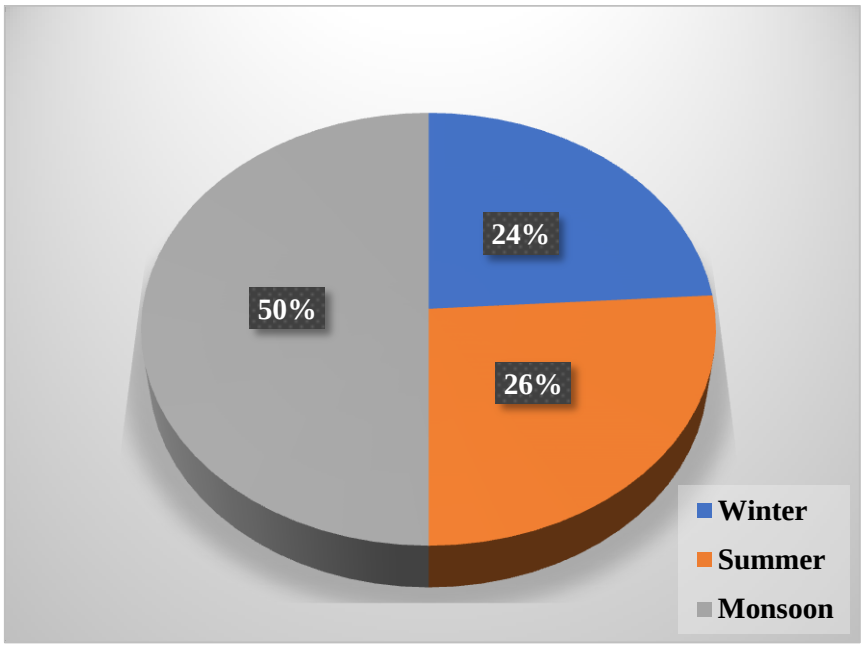

Figure 4. Season wise distribution of *B. bengalensis* collected from railway station

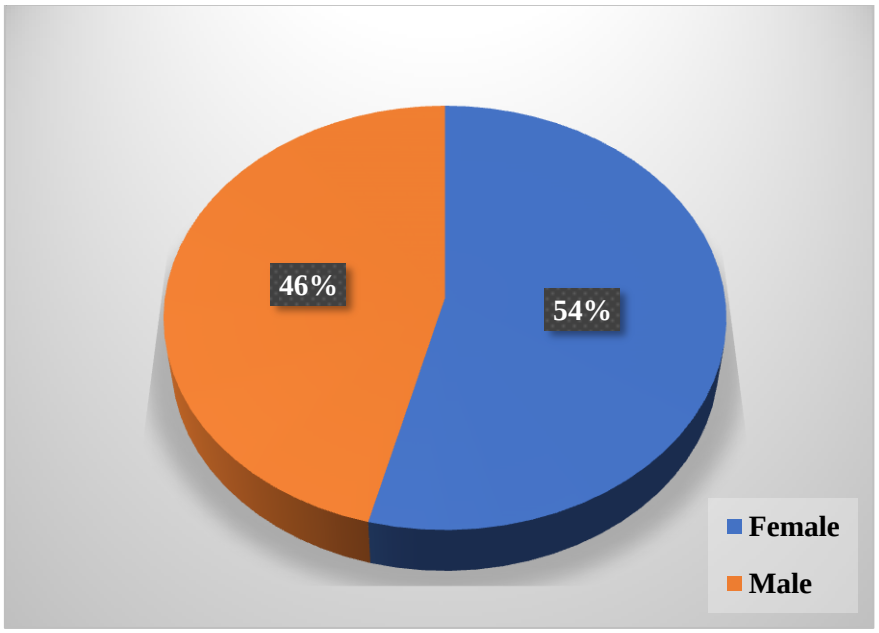

Figure 5. Sex wise distribution of *B. bengalensis* collected from railway station

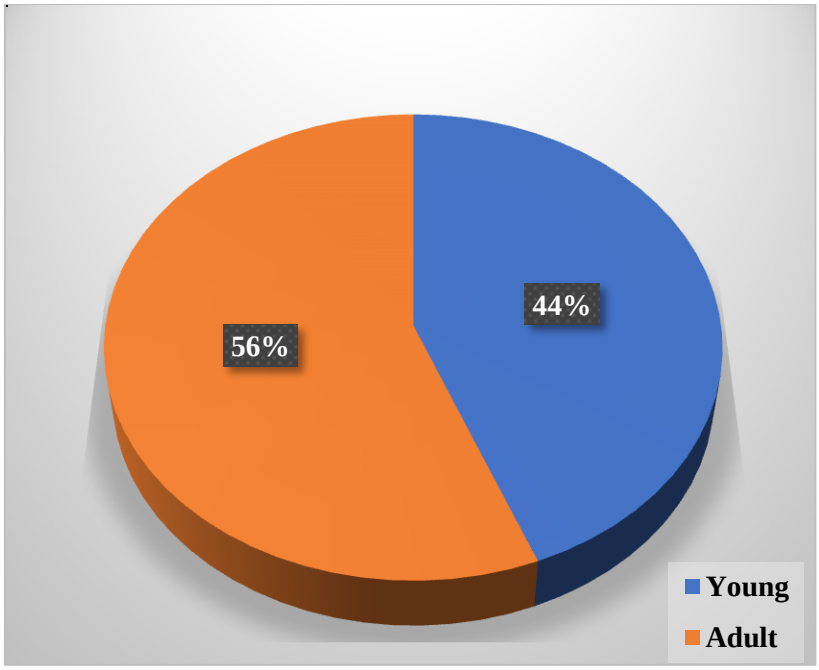

Figure 6. Age wise distribution of *B. bengalensis* collected from railway station

4.2 Analysis of rats for presence of ectoparasites

4.2.1 Analysis of rats collected from fish market

Out of total 50 rats collected from fish market and examined for the presence of ectoparasites, 28.00% (14 rats) were found infected with only one species of ectoparasites, i.e., Oriental rat flea, *X. cheopis*. Its season, host sex and age wise distribution is presented herewith.

4.2.1.1 Season wise distribution of ectoparasites

Out of total 27 rats collected and examined in winter season, 33.33% (9) were found infected with ectoparasites. While 27.27% (3/11) were found infected in summer and 16.67% (2/12) in monsoon seasons (Table 9). This predicted higher rate of ectoparasitic infestation and relative risk of disease transmission in winter season followed by summer and monsoon seasons (Figure 7).

Table 9. Season wise distribution of ectoparasitic infection in *B. bengalensis* collected from fish market

Status/season	Winter	Summer	Monsoon	Overall
Infected	9(33.33)	3(27.27)	2(16.67)	14(28.00)
Non-infected	18(66.67)	8(72.73)	10(83.33)	36(72.00)
Total	**27(100.00)**	**11(100.00)**	**12(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

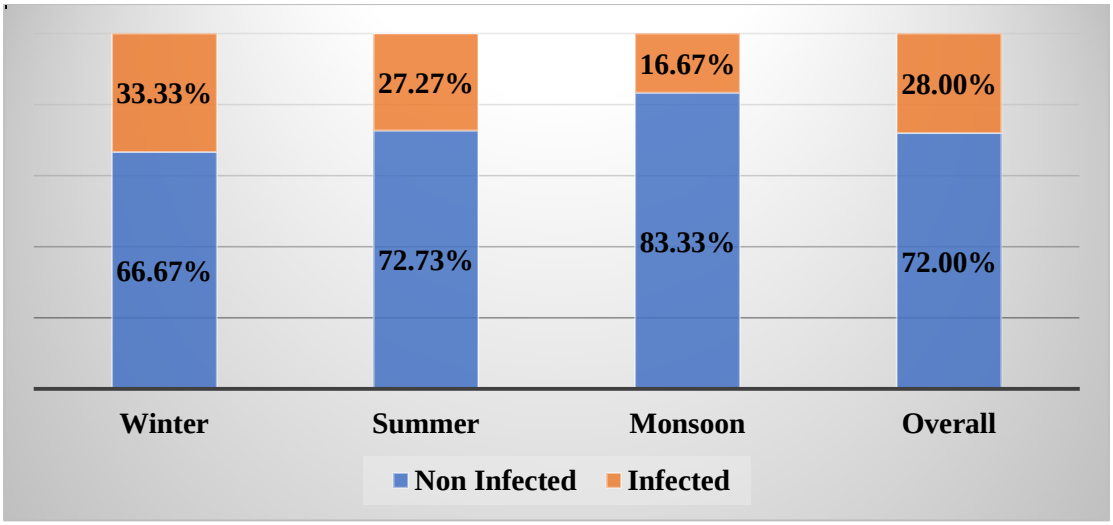

Figure 7. Season wise distribution of ectoparasitic infection in *B. bengalensis* collected from fish market

4.2.1.2 Host sex wise distribution of ectoparasites

Out of 33 female and 17 male rats examined, 30.30% female (10) and 23.53% male (4) rats were found infected with ectoparasites. This predicted higher rate of ectoparasitic infestation in females than that in males (Table 10) indicating relatively higher risk of disease transmission from female rats as compared to male rats (Figure 8).

Table 10. Host sex wise distribution of ectoparasitic infection in *B. bengalensis* collected from fish market

Status/sex	Female	Male	Overall
Infected	10(30.30)	4(23.53)	14(28.00)
Non-infected	23(69.70)	13(76.47)	36(72.00)
Total	**33(100.00)**	**17(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

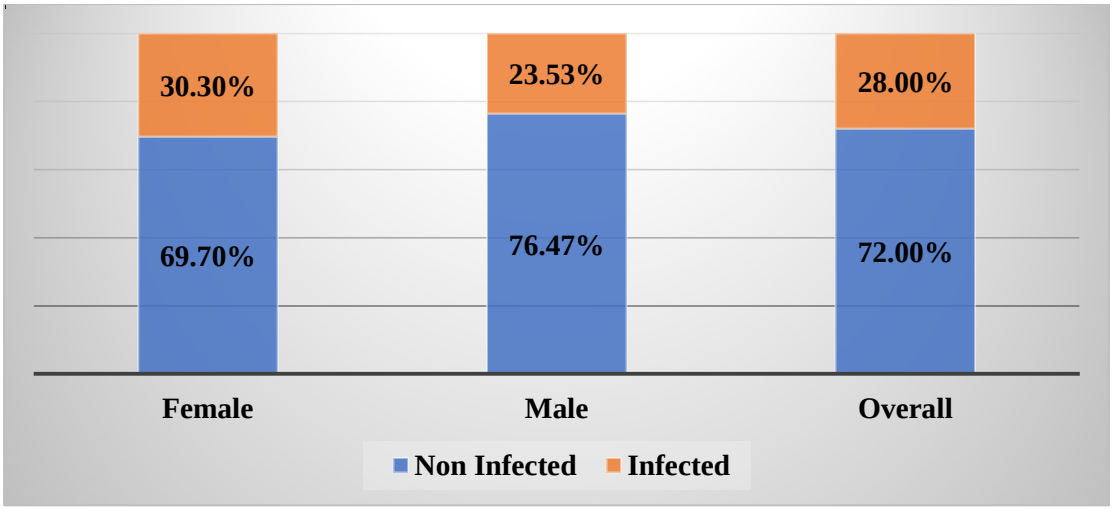

Figure 8. Host sex wise distribution of ectoparasitic infection in *B. bengalensis* collected from fish market

4.2.1.3 Host age wise distribution of ectoparasites

Out of 30 young and 20 adult rats examined, 23.33% young (7) and 35.00% adult (7) rats were found infected with ectoparasites. This predicted slightly higher rate of ectoparasitic infestation in adult individuals than that in young individuals (Table 11) indicating relatively higher risk of disease transmission from adult rats as compared to young rats (Figure 9).

Table 11. Host age wise distribution of ectoparasitic infection in *B. bengalensis* collected from fish market

Status/age	Young	Adult	Overall
Infected	7(23.33)	7(35.00)	14(28.00)
Non-infected	23(76.67)	13(65.00)	36(72.00)
Total	**30(100.00)**	**20(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

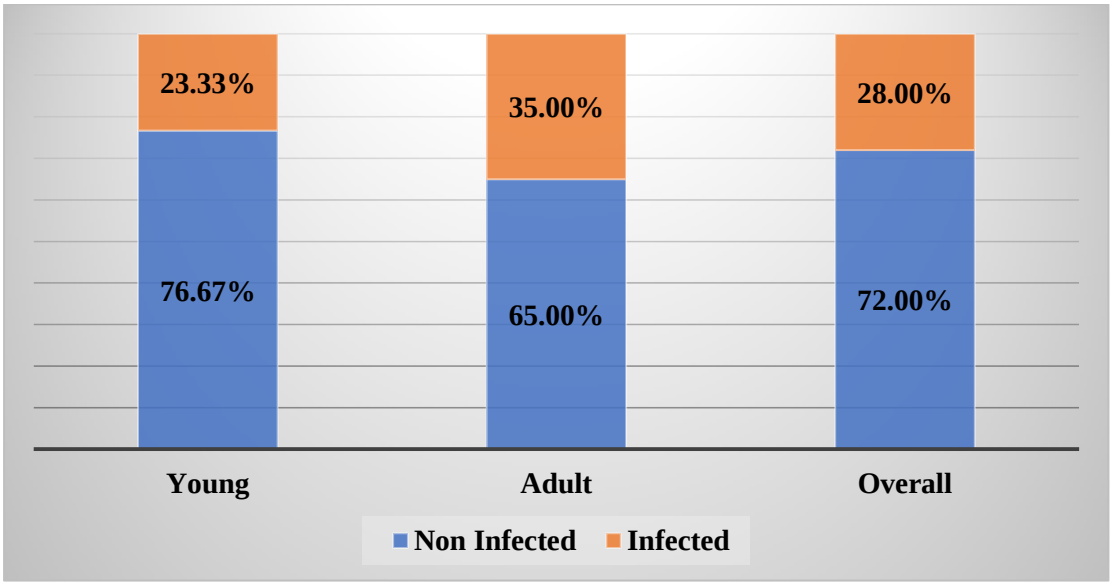

Figure 9. Host age wise distribution of ectoparasitic infection in *B. bengalensis* collected from fish market

4.2.1.4 Risk factor analysis for ectoparasitic infection found in rats collected from fish market

Total 18 oriental rat fleas were collected from 14 rats infected with ectoparasitic infection. Percent infection was high in adult (35.00%) female (30.30%) rats in winter season (33.33%). The parasite prevalence was also high in adult (55.56%) female (66.67%) rats in winter season (66.67%). The parasite intensity was, however, high in adult (1.43) male (1.50) rats in winter and summer (1.33) seasons. The parasite index, an indicator of risk factor analysis was high in adult (0.50) female (0.36) rats in winter (0.44) season indicating their involvement in disease transmission (Table 12). Overall, mean parasite intensity and parasite index were 1.29 and 0.36, respectively.

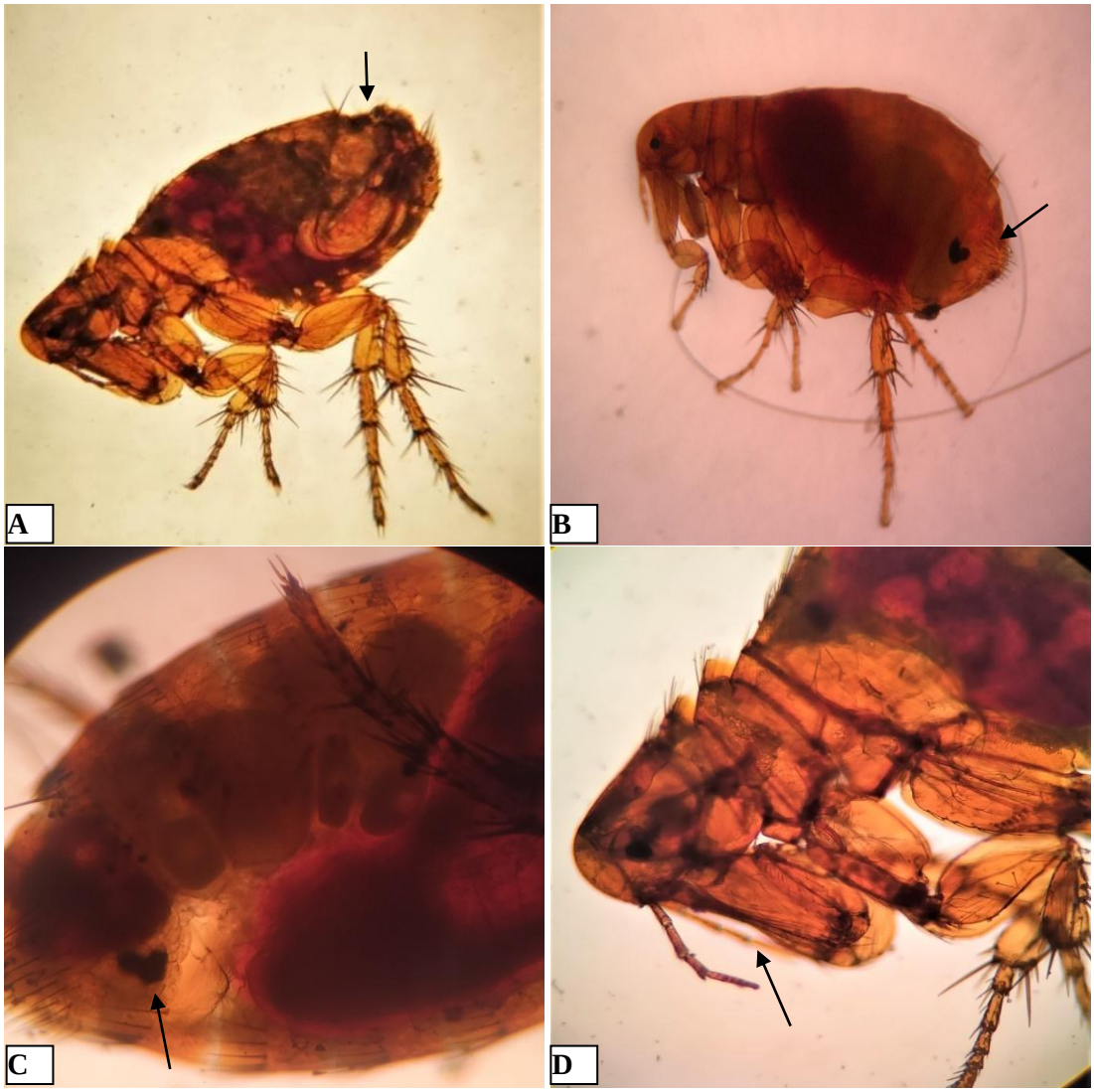

Plate 1. ***Xenopsylla cheopis*** **found in the fur of** ***Bandicota bengalensis,*** **A) Male rat flea showing long posterior legs and pygidium on the upper posterior end (arrow), B & C) Female rat flea with spermatheca (arrow), and D) Mouth parts of rat flea (arrow)**

Table 12. Variation in ectoparasitic infection in *B. bengalensis* collected from fish market in response to epidemiological factors

Epidemiological factors	Variable	Host examined	Host infected (% infected)	Relative risk	Number of fleas	Parasite prevalence (%)	Parasite intensity	Parasite index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	30	7 (23.33)	1.00	8	44.44	1.14	0.26	0.81	0.37 (1)	0.40	2.50
	Adult	20	7 (35.00)	1.50	10	55.56	1.43	0.50			0.62	3.62
Sex	Female	33	10(30.30)	1.28	12	66.67	1.20	0.36	0.25	0.61 (1)	0.47	3.50
	Male	17	4 (23.53)	1.00	6	33.33	1.50	0.35			0.29	3.36
Season	Winter	27	9 (33.33)	1.20	12	66.67	1.33	0.44	1.15	0.56 (2)	0.51	7.90
	Summer	11	3 (27.27)	1.64	4	22.22	1.33	0.36			0.33	8.03
	Monsoon	12	2 (16.67)	1.00	2	11.11	1.00	0.16			0.16	6.00
Overall		**50**	**14(28.00)**	**-**	**18**	**-**	**1.29**	**0.36**	**-**	**-**	**-**	**-**

Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of rat flea infestation in rats collected from fish market (Table 12).

4.2.2 Analysis of rats collected from railway station

Out of total 50 rats collected from railway station and examined for the presence of ectoparasites, 22.00% (11 rats) were found infected with only one species of ectoparasites, i.e., Oriental rat flea, *X. cheopis*. Its season, host sex and age wise distribution is presented herewith.

4.2.2.1 Season wise distribution of ectoparasites

Out of total 12 rats collected and examined in winter season, 33.33% (4) were found infected with ectoparasites. While 30.77% (4/13) were found infected in summer and 12.00% (3/25) in monsoon seasons (Table 13). This predicted higher rate of ectoparasitic infestation and relative risk of disease transmission in winter season followed by summer and monsoon seasons (Figure 10).

Table 13. Season wise distribution of ectoparasitic infection in *B. bengalensis* collected from railway station

Status/season	Winter	Summer	Monsoon	Overall
Infected	4(33.33)	4(30.77)	3(12.00)	11(22.00)
Non-infected	8(66.67)	9(69.23)	22(88.00)	39(78.00)
Total	**12(100.00)**	**13(100.00)**	**25(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

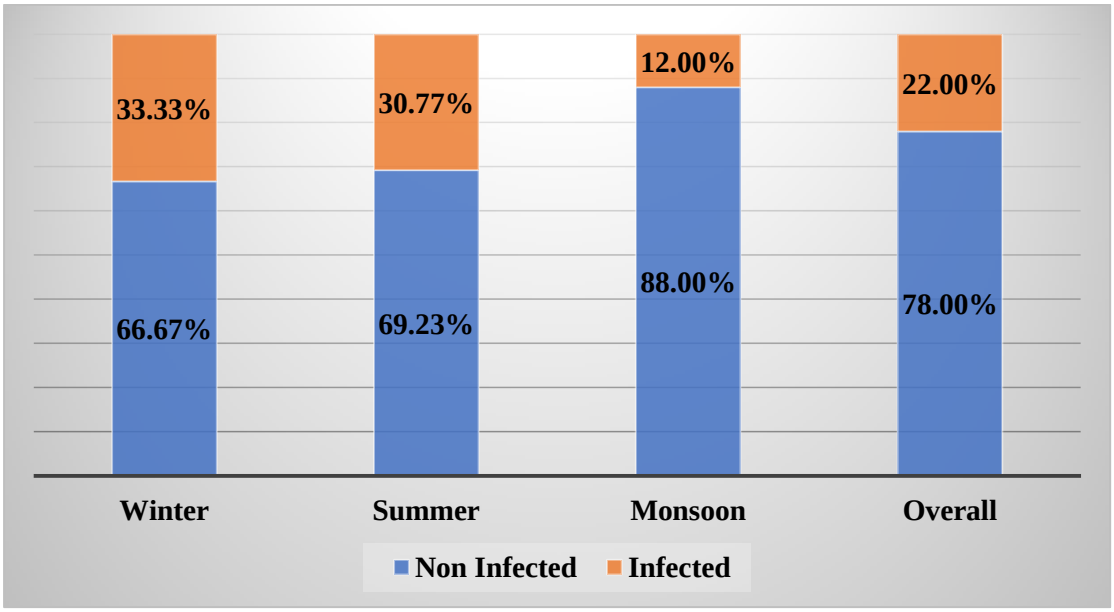

Figure 10. Season wise distribution of ectoparasitic infection in *B. bengalensis* collected from railway station

4.2.2.2 Host sex wise distribution of ectoparasites

Out of 27 female and 23 male rats examined, 33.33% female (9) and 8.70% male (2) rats were found infected with ectoparasites. This predicted higher rate of ectoparasitic infestation in females than that in males (Table 14) indicating relatively higher risk of disease transmission from female rats as compared to male rats (Figure 11).

Table 14. Host sex wise distribution of ectoparasitic infection in *B. bengalensis* collected from railway station

Status/sex	Female	Male	Overall
Infected	9(33.33)	2(8.70)	11(22.00)
Non-infected	18(66.67)	21(91.30)	39(78.00)
Total	**27(100.00)**	**23(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

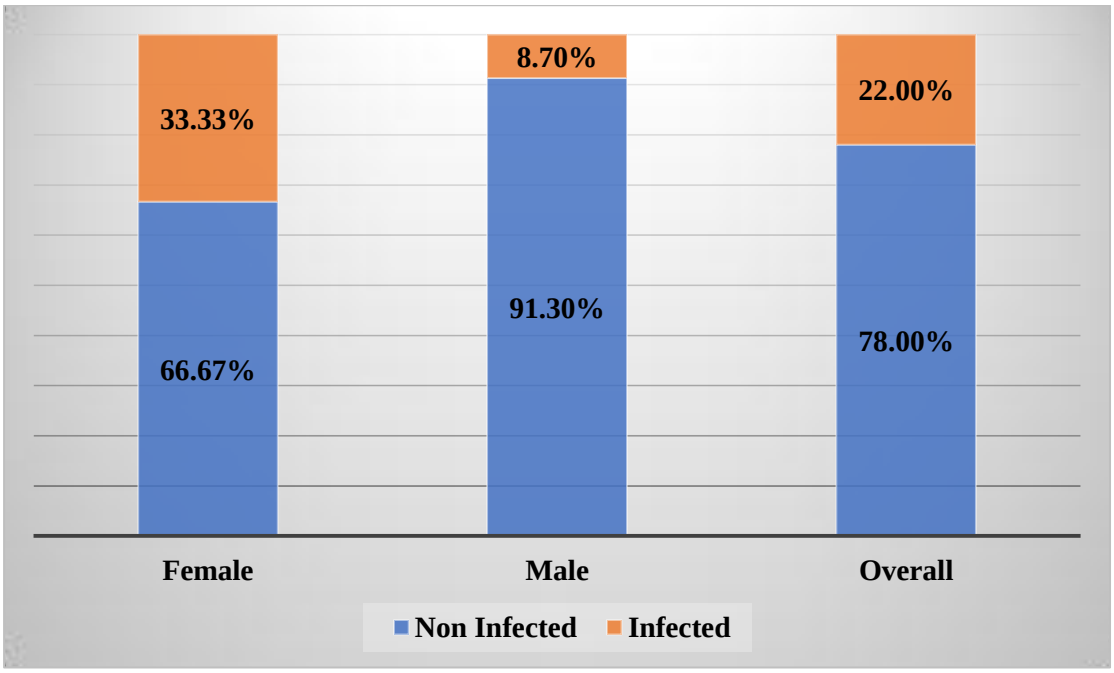

Figure 11. Host sex wise distribution of ectoparasitic infection in *B. bengalensis* collected from railway station

4.2.2.3 Host age wise distribution of ectoparasites

Out of 22 young and 28 adult rats examined, 22.73% young (5) and 21.43% adult (6) rats were found infected with ectoparasites. This predicted almost similar rate of ectoparasitic infestation in young and adult individuals (Table 15) indicating relatively similar risk of disease transmission from both young and adult rats (Figure 12).

Table 15. Host age wise distribution of ectoparasitic infection in *B. bengalensis* collected from railway station

Status/age	Young	Adult	Overall
Infected	5(22.73)	6(21.43)	11(22.00)
Non-infected	17(77.27)	22(78.57)	39(78.00)
Total	**22(100.00)**	**28(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

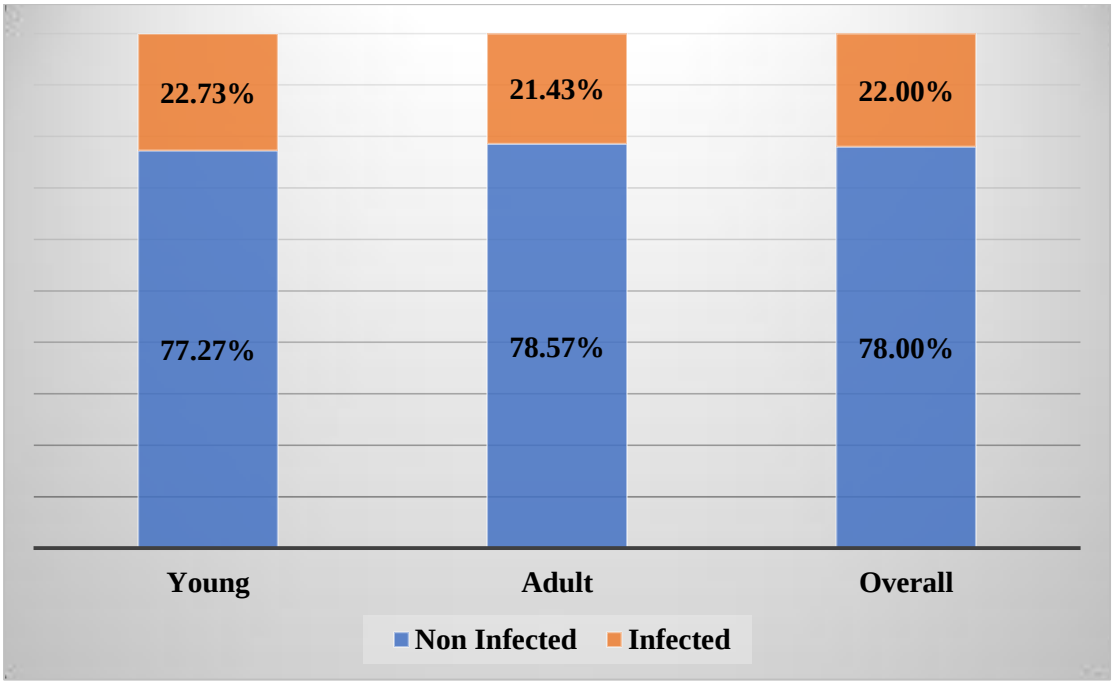

Figure 12: Host age wise distribution of ectoparasitic infection in *B. bengalensis* collected from railway station

4.2.2.4 Risk factor analysis for ectoparasitic infection found in rats collected from railway station

Total 16 oriental rat fleas were collected from 11 rats infected with ectoparasitic infection. Percent infection was high in young (22.73%) female (33.33%) rats in winter season (33.33%). The parasite prevalence was same in both young and adult (50.00%) individuals. It was high in female (81.25%) rats in summer season (43.75%). The parasite intensity was, however, high in young (1.60) male (1.50) rats in summer (1.75) season. The parasite index, an indicator of risk factor analysis was high in young (0.36) female (0.48) rats in summer (0.54) season indicating their involvement in disease transmission (Table 16). Overall, mean parasite intensity and parasite index were 1.45 and 0.32, respectively.

Statistical analysis of the data revealed that host sex had significant (χ^2 = 4.39, P = 0.0361 and df = 1) effect on the prevalence of rat lea infestation, while the host age and season had no significant (P>0.05) effect on rat flea infestation (Table 16).

Table 16. Variation in ectoparasitic infection in *B. bengalensis* collected from railway station in response to epidemiological factors

Epidemiological factors	Variable	Host examined	Host infected (% infected)	Relative risk	Number of fleas	Parasite prevalence	Parasite intensity	Parasite index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	22	5 (22.73)	1.06	8	50.00	1.60	0.36	0.01	0.91 (1)	0.37	3.02
	Adult	28	6(21.43)	1.00	8	50.00	1.33	0.29			0.40	2.73
Sex	Female	27	9(33.33)	3.83	13	81.25	1.44	0.48	4.39	0.0361 (1)	0.92	15.98
	Male	23	2(8.70)	1.00	3	18.75	1.50	0.13			0.15	6.50
Season	Winter	12	4(33.33)	2.78	5	31.25	1.25	0.42	1.42	0.70 (2)	0.73	10.50
	Summer	13	4(30.77)	2.56	7	43.75	1.75	0.54			0.67	9.77
	Monsoon	25	3(12.00)	1.00	4	25.00	1.33	0.16			0.22	4.49
Overall		**50**	**11(22.00)**	-	**16**	-	**1.45**	**0.32**	-	-	-	-

4.2.3 Location wise comparison of ectoparasitic infection

Total 100 rats (50 each) were collected from two locations. In overall, 25.00% rats were found infected with rat fleas. Total 34 rat fleas were collected from 25 rats with mean parasite intensity of 1.36 and mean parasite index of 0.34 (Table 17).

Table 17. Location wise comparison of ectoparasitic infection in *B. bengalensis*

Location	Host examined	Host infected	Host non-infected	Percent host infected	Number of fleas	Parasite prevalence	Parasite intensity	Parasite index
Fish market	50	14	36	28.00	18	52.94	1.29	0.36
Railway station	50	11	39	22.00	16	47.06	1.45	0.32
Overall	**100**	**25**	**75**	**25.00**	**34**	**-**	**1.36**	**0.34**

4.2.4 Morphological features of *X. cheopis* and zoonotic potential

The rat flea, *X. cheopis* of both sexes was found on *B. bengalensis* in present study. Female rat fleas had a characteristic spermatheca, while both the sexes had a pygidium at the upper posterior end. Body was segmented and posterior legs were long (Plate 1). In present study, more infection of rat flea was found in winter and summer season as compared to monsoon season. Nieto *et al* (2007) observed that flea infestation was more in summer and spring as compared to fall and winter. The rat flea, *X. cheopis* found in present study is the most important vector of plague and the rickettsial infection of murine typhus (Gratz 1999). Dennis *et al* (1999) and Moore and Gage (2005) used flea index to estimate human and epizootic risk for plague. They correlated flea index>1.0 with increase in plague risk to humans. In present study, the overall flea index was found to be <1.0 (0.34) with infection rate of 25.00% in *B. bengalensis* collected from two commensal locations indicating low risk of disease transmission.

4.3 Analysis of rats for presence of endoparasites

4.3.1 Analysis of rats collected from fish market

4.3.1.1 Overall analysis of endoparasites found in rats collected from fish market

Out of total 50 rats collected from fish market and examined for the presence of endoparasites, 86.00% (43 rats) were found infected with one or more species of endoparasites. Their season, host sex and age wise distribution is presented herewith.

4.3.1.1.1 Season wise distribution of endoparasites

All the total 27 rats (100.00%) collected and examined in winter season were found infected with endoparasites. While seven (63.63%) and nine (75.00%) were found infected in summer and monsoon seasons, respectively (Table 18). This predicted higher rate of

endoparasitic infestation and relative risk of disease transmission in winter season followed by monsoon and summer seasons (Figure 13).

Table 18. Season wise distribution of endoparasitic infection in *B. bengalensis* collected from fish market

Status/season	Winter	Summer	Monsoon	Overall
Infected	27(100.00)	7(63.63)	9(75.00)	43(86.00)
Non-infected	-	4(36.36)	3(25.00)	7(14.00)
Total	**27(100.00)**	**11(100.00)**	**12(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

4.3.1.1.2 Host sex wise distribution of endoparasites

Out of 33 female and 17 male rats examined, 81.82% female (27) and 94.12% male (16) rats were found infected with endoparasites. This predicted higher rate of endoparasitic infestation in males than that in females (Table 19) indicating relatively higher risk of disease transmission from male rats as compared to female rats (Figure 14).

Table 19. Host sex wise distribution of endoparasitic infection in *B. bengalensis* collected from fish market

Status/sex	Female	Male	Overall
Infected	27(81.82)	16(94.12)	43(86.00)
Non-infected	6(18.18)	1(5.88)	7(14.00)
Total	**33(100.00)**	**17(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

4.3.1.1.3 Host age wise distribution of endoparasites

Out of 30 young and 20 adult rats examined, 26 young (86.67%) and 17 adult (85.00%) rats were found infected with endoparasites. This predicted slightly higher rate of endoparasitic infestation in young individuals than that in adults (Table 20) indicating relatively higher risk of disease transmission from young rats as compared to adults (Figure 15).

Table 20. Host age wise distribution of endoparasitic infection in *B. bengalensis* collected from fish market

Status/age	Young	Adult	Overall
Infected	26(86.67)	17(85.00)	43(86.00)
Non-infected	4(13.33)	3(15.00)	7(14.00)
Total	**30(100.00)**	**20(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

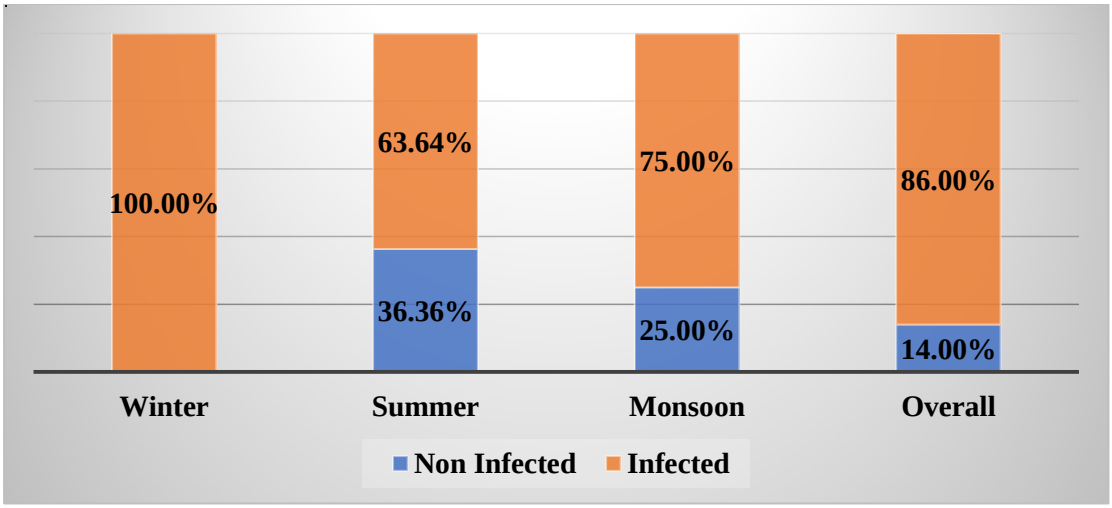

Figure 13. Season wise distribution of endoparasitic infection in *B. bengalensis* collected from fish market

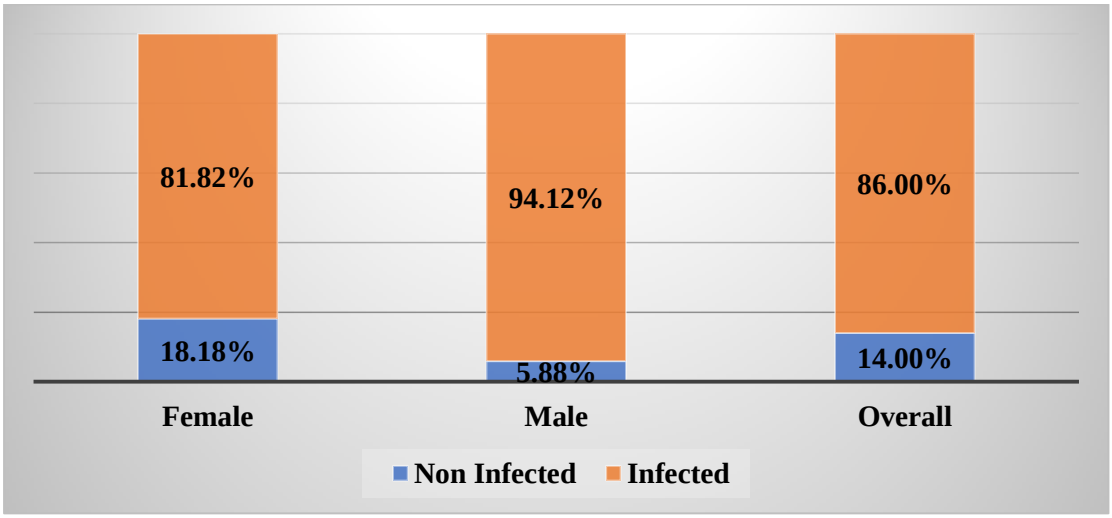

Figure 14. Host sex wise distribution of endoparasitic infection in *B. bengalensis* collected from fish market

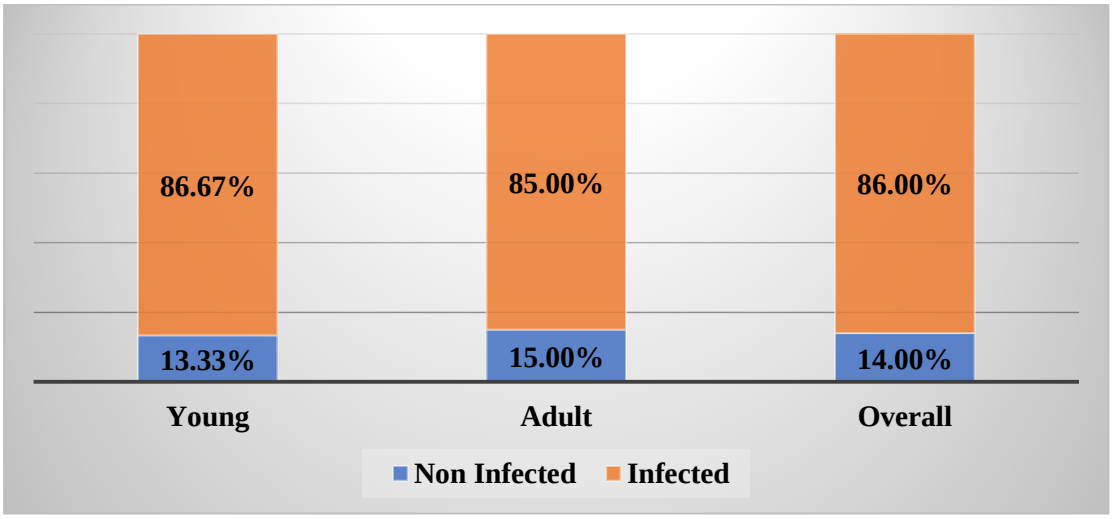

Figure 15. Host age wise distribution of endoparasitic infection in *B. bengalensis* collected from fish market

4.3.1.2 Individual analysis of endoparasites found in rats collected from fish market

Out of total 50 rats examined, 86.00% (43) were found infected with total seven species of endoparasites, comprising two species of cestodes i.e., *H. nana* and *C. fasciolaris* and five species of nematodes i.e., *N. brasiliensis, C. hepaticum, S. muris, T. muris* and *H. spumosa*. The *H. nana* was found in the small intestine and *C. fasciolaris* was found in the liver in the form of whitish cysts. The nematode parasites were found at multiple locations in the host like *C. hepaticum* was found in liver. Infection was identified based on yellowish white streaks or spots on the surface of liver. *N. brasiliensis* was found in small intestine, *S. muris* and *H. spumosa* were found in large intestine, whereas, *T. muris* was found specifically in caecum (Table 21). Rats were found infected with one or more number of parasite species simultaneously indicating concurrent infection (Table 22). Prevalence, intensity and risk factor analysis of all the endoparasites is presented herewith individually.

Table 21. Site of infection of different endoparasites found in *B. bengalensis*

Class of endoparasites	Endoparasites	Site of infection
Cestoda	*H. nana*	Small intestine
	C. fasciolaris	Liver
Nematoda	*N. brasiliensis*	Small intestine
	C. hepaticum	Liver
	S. muris	Large intestine
	T. muris	Large intestine (Caecum)
	H. spumosa	Large intestine

Table 22. Overall infection of endoparasites in *B. bengalensis* collected from fish market

Class of endoparasites	Name of endoparasite	Host infected (n = 50)	
		Number	Percentage
Cestoda	*H. nana*	17	34.00
	C. fasciolaris	6	12.00
Nematoda	*N. brasiliensis*	24	48.00
	C. hepaticum	23	46.00
	S. muris	7	14.00
	T. muris	13	26.00
	H. spumosa	10	20.00
Overall		**100***	**-**

*Concurrent infection in rats with one or more endoparasite species

4.3.1.2.1 Analysis of cestode parasites

4.3.1.2.1.1 Analysis of *H. nana*

Out of total 50 rats, 17.00% (34) were found infected with *H. nana*. Percent infection was higher in winter (51.85%) followed by summer (18.18%) and monsoon (8.33) seasons. Similarly, percent infection was higher in male (34.18%) and adult rats (35.00%) indicating higher relative risk of disease transmission from adult and male rats in winter season as compared to young and female rats in summer and monsoon seasons (Figures 16-18). Total 117 numbers of parasites were recovered from 34 rats with higher prevalence of infection in young rats (51.28%) compared to adult (48.72%), female rats (61.54%) compared to male rats (38.46%) and winter season (94.87%). Percent parasite prevalence was very low in summer and monsoon seasons. The parasite intensity and parasite index were high in adult rats and male rats in winter season. Overall mean parasite intensity and parasite index were 6.88 and 2.34, respectively. Statistical analysis of the data revealed that season had significant (χ^2 = 8.58, P = 0.0137 and df = 2) effect on the prevalence of *H. nana* infection while host age and sex had no significant (P >0.05) effect (Table 23).

4.3.1.2.1.2 Analysis of *C. fasciolaris*

Out of total 50 rats, 12.00% (6) were found infected with *C. fasciolaris*. Percent infection was higher in monsoon (16.67%) followed by winter (11.11%) and summer (9.09%) seasons. Similarly, percent infection was higher in male (17.64%) and adult rats (15.00%) indicating higher relative risk of disease transmission from adult and male rats in monsoon season as compared to young and female rats in winter and summer seasons (Figures 19-21). Total six numbers of parasites were recovered from 6 rats (one cyst in each rat). Similar prevalence of infection was observed in young and adult rats (50.00%) as well as in female and male rats (50.00%). Prevalence of parasites was higher in winter season (50.00%) compared to monsoon (33.00%) and summer (17.00%) seasons. The parasite intensity was high in adult rats and male rats in monsoon season. Overall mean parasite intensity and parasite index were 0.12 and 1.00, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P >0.05) effect on the prevalence of *C. fasciolaris* infection (Table 24).

4.3.1.2.1.3 Overall analysis of cestodes

Out of total 50 rats, 42.00% (21) were found infected with two species of cestodes. Percent infection was higher in winter (55.55%) followed by summer (27.27%) and monsoon (25.00%) seasons. Similarly, percent infection was higher in male (52.94%) and young rats (43.33%) indicating higher relative risk of disease transmission from young and female rats in winter season as compared to adult and female rats in summer and monsoon seasons (Figures

Table 23. Risk factor analysis of *H. nana* infection in *B. bengalensis* collected from fish market

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	30	10(33.33)	1.00	60	51.28	6.00	2.00	0.01	0.90 (1)	0.48	2.29
	Adult	20	7(35.00)	1.05	57	48.72	8.14	2.85			0.48	2.04
Sex	Female	33	10(30.30)	1.00	72	61.54	7.20	2.18	0.59	0.44 (1)	0.39	2.06
	Male	17	7(41.18)	1.36	45	38.46	6.42	2.64			0.40	2.83
Season	Winter	27	14(51.85)	6.22	111	94.87	7.92	4.11	8.58	0.0137 (2)	0.92	42.07
	Summer	11	2(18.18)	2.18	5	4.27	2.50	0.45			0.22	20.84
	Monsoon	12	1(8.33)	1.00	1	0.01	1.00	0.08			0.07	14.20
Overall		**50**	**17(34.00)**	-	**117**	-	**6.88**	**2.34**	-	-	-	-

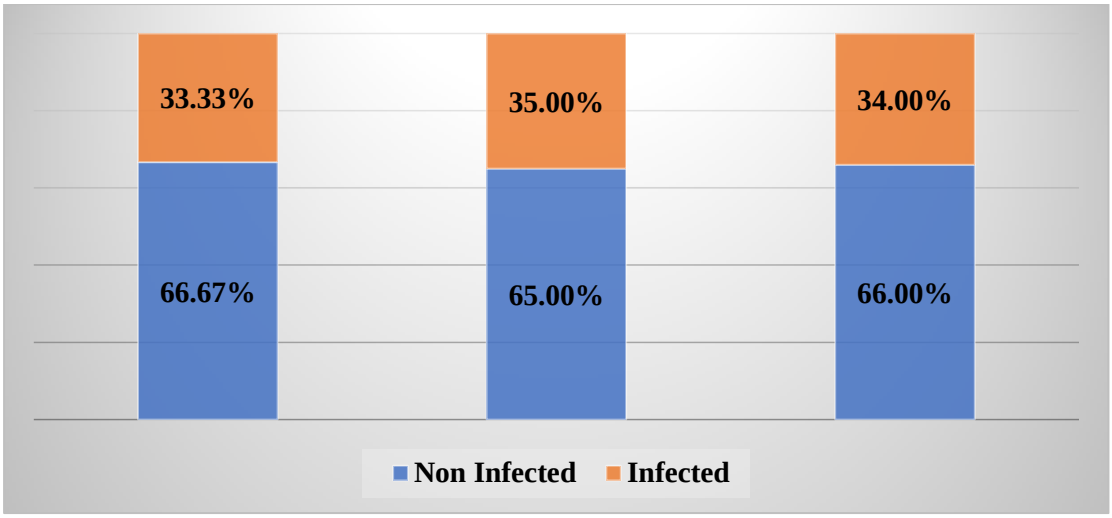

Figure 16. Host age wise distribution of *H. nana* infection in *B. bengalensis* collected from fish market

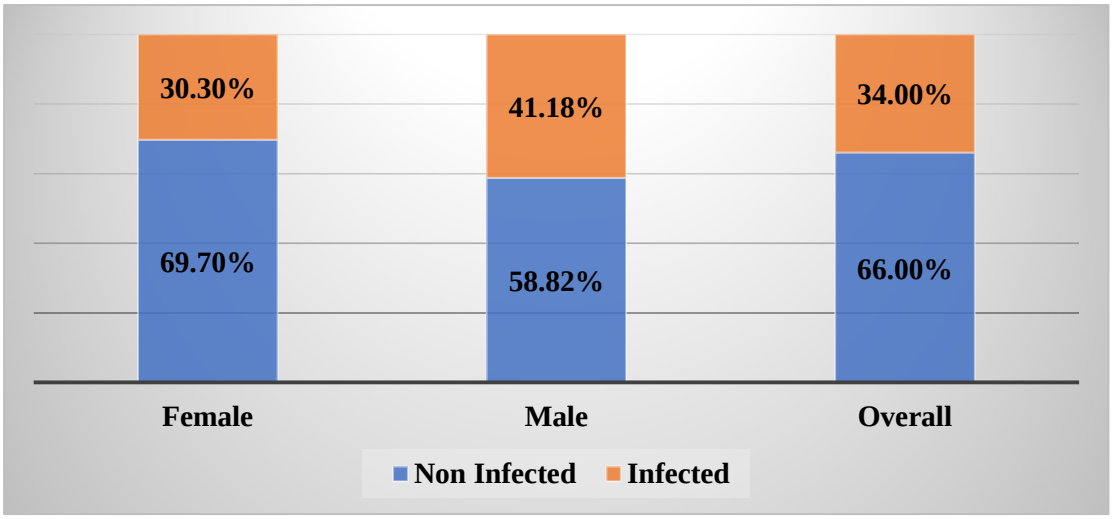

Figure 17. Host sex wise distribution of *H. nana* infection in *B. bengalensis* collected from fish market

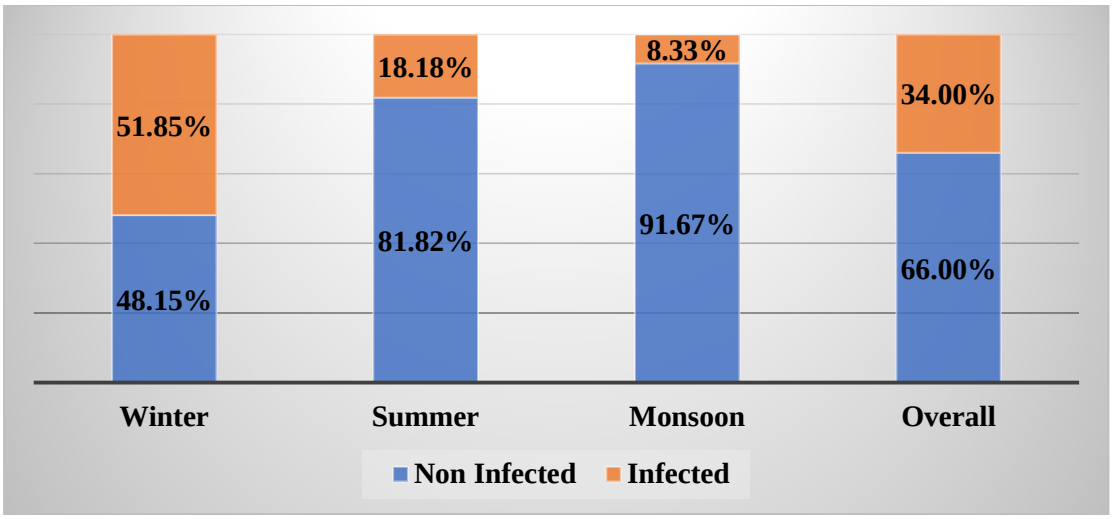

Figure 18. Season wise distribution of *H. nana* infection in *B. bengalensis* collected from fish market

Table 24. Risk factor analysis of *C. fasciolaris* infection in *B. bengalensis* collected from fish market

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	30	3(10.00)	1.00	3	50.00	1.00	0.10	0.28	0.59 (1)	0.23	6.70
	Adult	20	3(15.00)	1.50	3	50.00	1.00	0.15			0.21	4.56
Sex	Female	33	3(9.09)	1.00	3	50.00	1.00	0.09	0.78	0.38 (1)	0.43	8.60
	Male	17	3(17.64)	1.78	3	50.00	1.00	0.18			0.21	4.59
Season	Winter	27	3(11.11)	1.22	3	50.00	1.00	0.11	0.36	0.84 (2)	0.14	10.51
	Summer	11	1(9.09)	1.00	1	17.00	1.00	0.09			0.07	14.05
	Monsoon	12	2(16.67)	1.68	2	33.00	1.00	0.17			0.19	17.51
Overall		**50**	**6(12.00)**	**-**	**6**	**-**	**1.00**	**0.12**	**-**	**-**	**-**	**-**

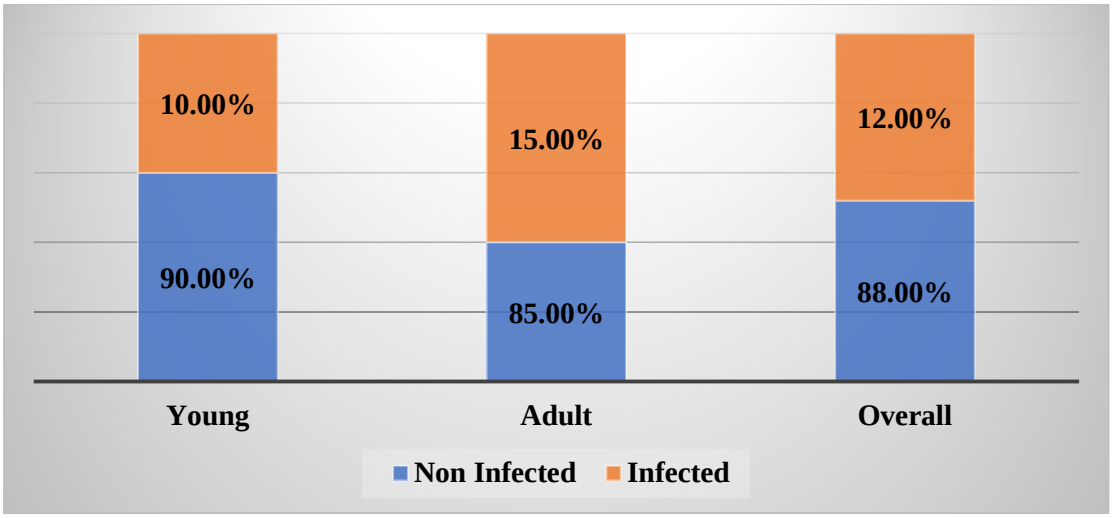

Figure 19. Host age wise distribution of *C. fasciolaris* infection in *B. bengalensis* collected from fish market

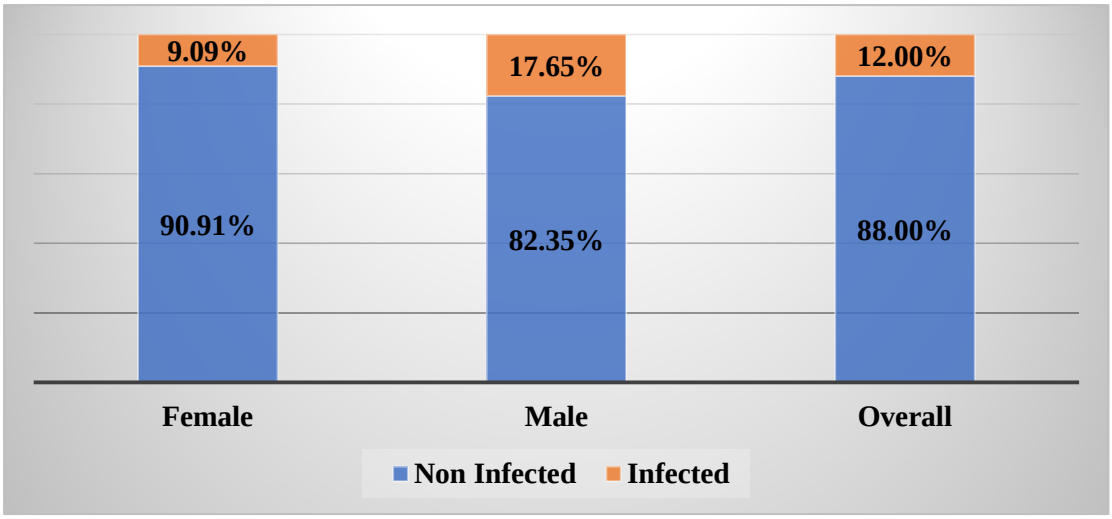

Figure 20. Host sex wise distribution of *C. fasciolaris* infection in *B. bengalensis* collected from fish market

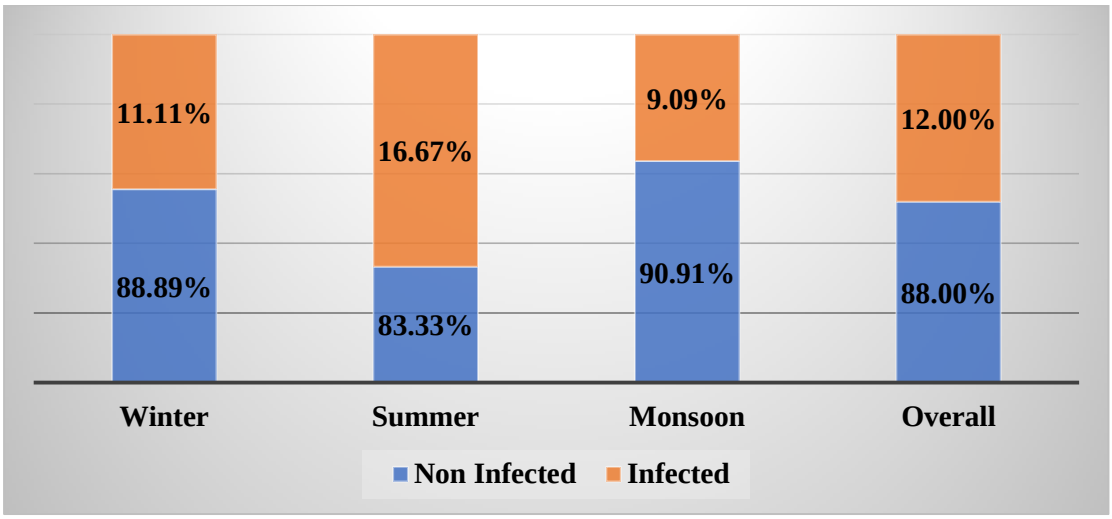

Figure 21. Season wise distribution of *C. fasciolaris* infection in *B. bengalensis* collected from fish market

22-24). Total 123 numbers of parasites were recovered from 21 rats. Prevalence of infection was higher in young rats (51.21%) and female rats (60.97%). Prevalence of parasites was higher in winter season (92.68%) compared to summer (4.87%) and monsoon (2.43%) seasons. The parasite intensity was high in adult rats and female rats in winter season. The parasite index was high in adult rats and male rats in winter season. Overall mean parasite intensity and parasite index were 5.85 and 2.46, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P >0.05) effect on the prevalence of overall cestode infection (Table 25).

4.3.1.2.2 Analysis of nematode parasites

4.3.1.2.2.1 Analysis of *N. brasiliensis*

Out of total 50 rats, 48.00% (24) were found infected with *N. brasiliensis*. Percent infection was higher in monsoon (50.00%) followed by winter (48.15%) and summer (45.45%) seasons. Similarly, percent infection was higher in male (64.71%) and young rats (53.33%) indicating higher relative risk of disease transmission from young and male rats in monsoon season as compared to adult and female rats in winter and summer seasons (Figures 25-27). Total 913 numbers of parasites were recovered from 24 rats. Prevalence of infection was more in young rats (70.10%) and female rats (54.43%). Prevalence of parasites was higher in winter season (73.82%) compared to summer (22.56%) and monsoon (3.61%) seasons. The parasite intensity was high in young rats and female rats in winter season, while the parasite index was high in young and male rats in winter season. Overall mean parasite intensity and parasite index were 38.04 and 18.26, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P >0.05) effect on the prevalence of *N. brasiliensis* infection (Table 26).

4.3.1.2.2.2 Analysis of *C. hepaticum*

Out of total 50 rats, 46.00% (23) were found infected with *C. hepaticum*. Percent infection was higher in monsoon (50.00%) followed by winter (48.15%) and summer (36.36%) seasons. Similarly, percent infection was higher in male (52.94%) and young rats (53.33%) indicating higher relative risk of disease transmission from young and male rats in monsoon season as compared to adult and female rats in winter and summer seasons (Figures 28-30). *C. hepaticum* infection was in the form of eggs enclosed in liver parenchyma. No adult worms were seen in the liver (Table 27).

Statistical analysis of the data revealed that host age, sex and season had no significant (P >0.05) effect on the prevalence of *C. hepaticum* infection (Table 27).

Table 25. Risk factor analysis of cestode infection in *B. bengalensis* collected from fish market

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	30	13(43.33)	1.08	63	51.21	4.84	**2.10**	0.05	0.81 (1)	0.55	2.12
	Adult	20	8(40.00)	1.00	60	48.78	7.50	**3.00**			0.46	2.13
Sex	Female	33	12(36.36)	1.00	75	60.97	6.25	**2.27**	1.26	0.26 (1)	0.52	1.89
	Male	17	9(52.94)	1.45	48	39.02	5.33	**2.66**			0.47	1.90
Season	Winter	27	15(55.55)	2.22	114	92.68	7.60	**4.22**	4.44	0.11 (2)	0.78	6.26
	Summer	11	3(27.27)	1.09	6	4.87	2.00	**0.54**			0.27	4.31
	Monsoon	12	3(25.00)	1.00	3	2.43	1.00	**0.25**			0.25	3.99
Overall		**50**	**21(42.00)**	-	**123**	-	**5.85**	**2.46**	-	-	-	-

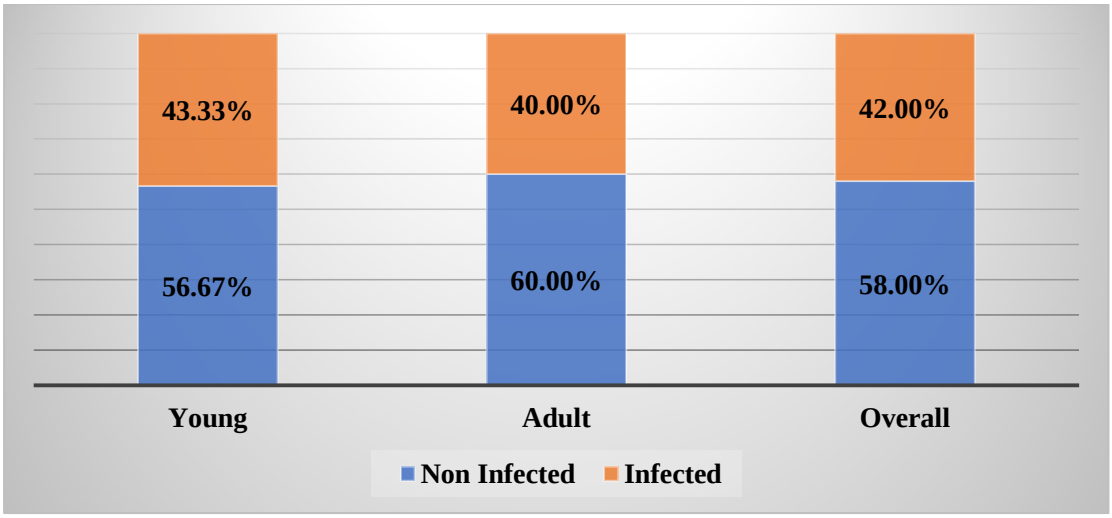

Figure 22. Host age wise distribution of cestode infection in *B. bengalensis* collected from fish market

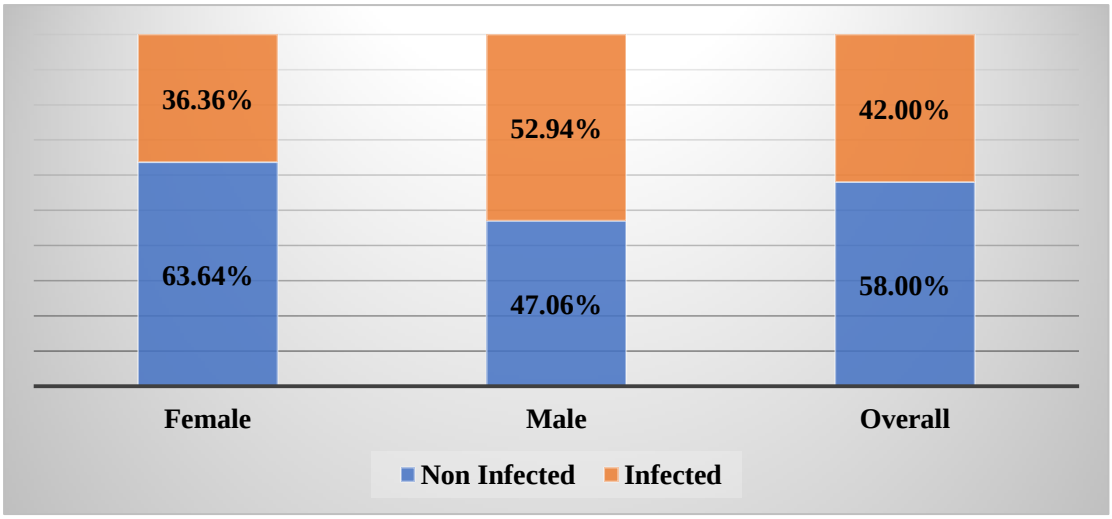

Figure 23. Host sex wise distribution of cestode infection in *B. bengalensis* collected from fish market

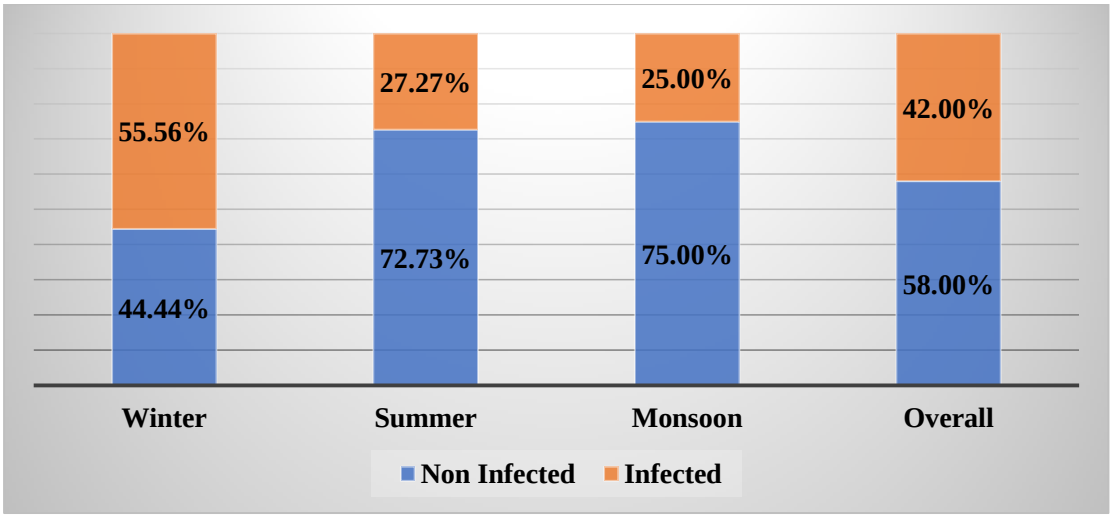

Figure 24. Season wise distribution of cestode infection in *B. bengalensis* collected from fish market

Table 26. Risk factor analysis of *N. brasiliensis* infection in *B. bengalensis* collected from fish market

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	30	16(53.33)	1.33	640	70.10	40.00	21.33	0.85	0.35 (1)	0.71	2.51
	Adult	20	8(40.00)	1.00	273	29.90	34.12	13.65			0.46	2.13
Sex	Female	33	13(39.39)	1.00	497	54.43	38.23	15.06	2.88	0.09 (1)	0.54	1.81
	Male	17	11(64.71)	1.64	416	45.56	37.82	24.47			0.94	2.90
Season	Winter	27	13(48.15)	1.06	674	73.82	51.85	24.96	0.05	0.98 (2)	0.49	2.25
	Summer	11	5(45.45)	1.00	206	22.56	41.20	18.73			0.40	2.49
	Monsoon	12	6(50.00)	1.10	33	3.61	5.50	2.75			0.46	2.59
Overall		**50**	**24(48.00)**	-	**913**	-	**38.04**	**18.26**	-	-	-	-

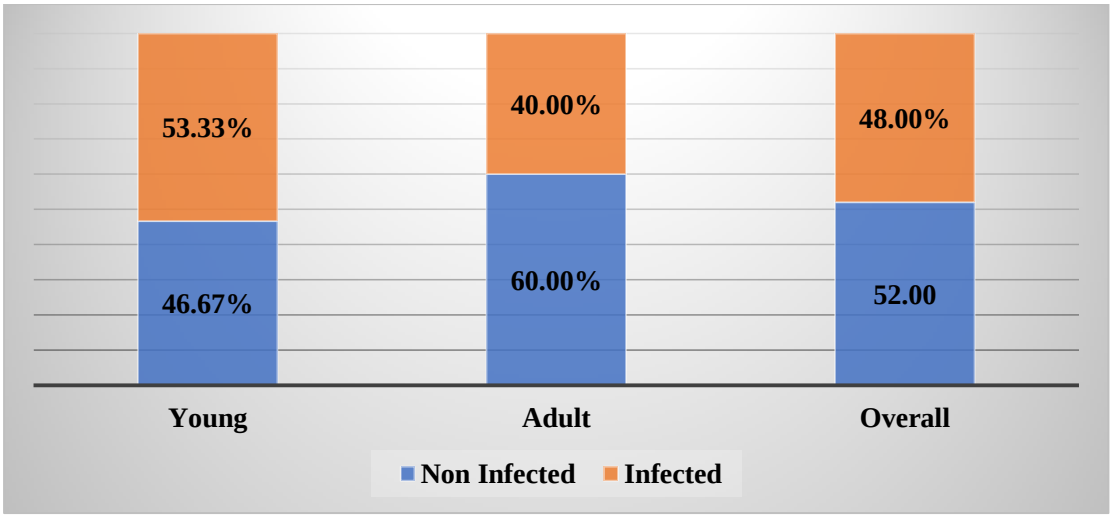

Figure 25. Host age wise distribution of infection *N. brasiliensis* in *B. bengalensis* collected from fish market

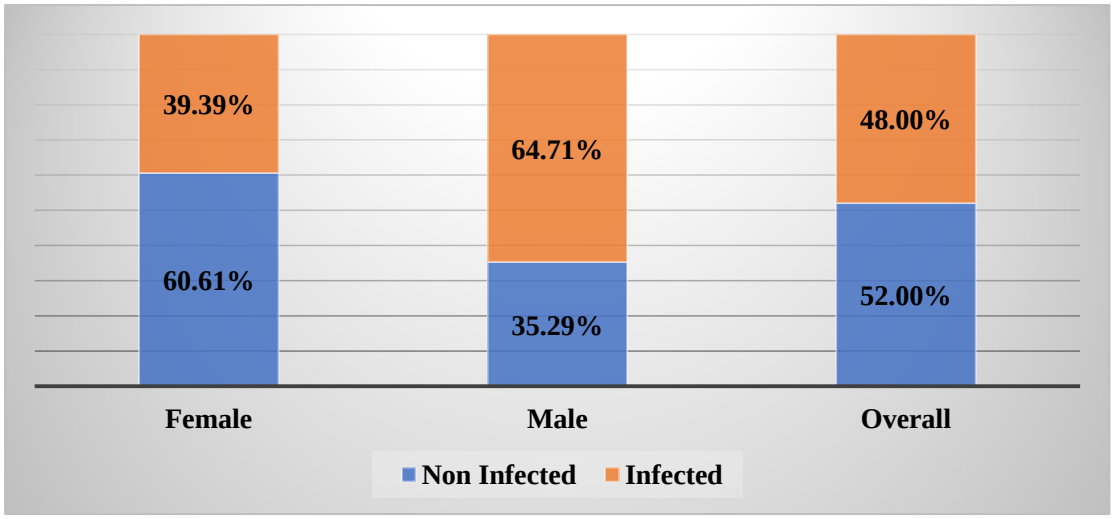

Figure 26. Host sex wise distribution of *N. brasiliensis* infection in *B. bengalensis* collected from fish market

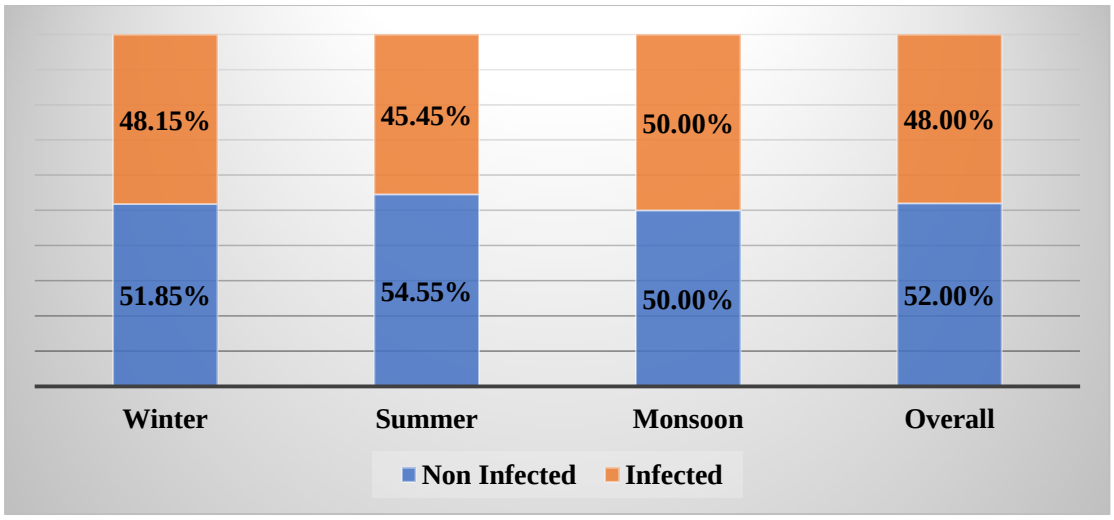

Figure 27. Season wise distribution of *N. brasiliensis* infection in *B. bengalensis* collected from fish market

Table 27. Risk factor analysis of *C. hepaticum* infection in *B. bengalensis* collected from fish market

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Chi square value	P value (d.f.)	95% Confidence interval	
							Lower limit	Upper limit
Age	Young	30	16(53.33)	1.52	1.62	0.20 (1)	0.76	3.02
	Adult	20	7(35.00)	1.00			0.42	2.32
Sex	Female	33	14(42.42)	1.00	0.50	0.48 (1)	0.48	2.27
	Male	17	9(52.94)	1.25			0.47	1.75
Season	Winter	27	13(48.15)	1.32	0.54	0.76 (2)	0.53	3.17
	Summer	11	4(36.36)	1.00			0.33	3.02
	Monsoon	12	6(50.00)	1.37			0.52	3.60
Overall		**50**	**23(46.00)**	-	-	-	-	-

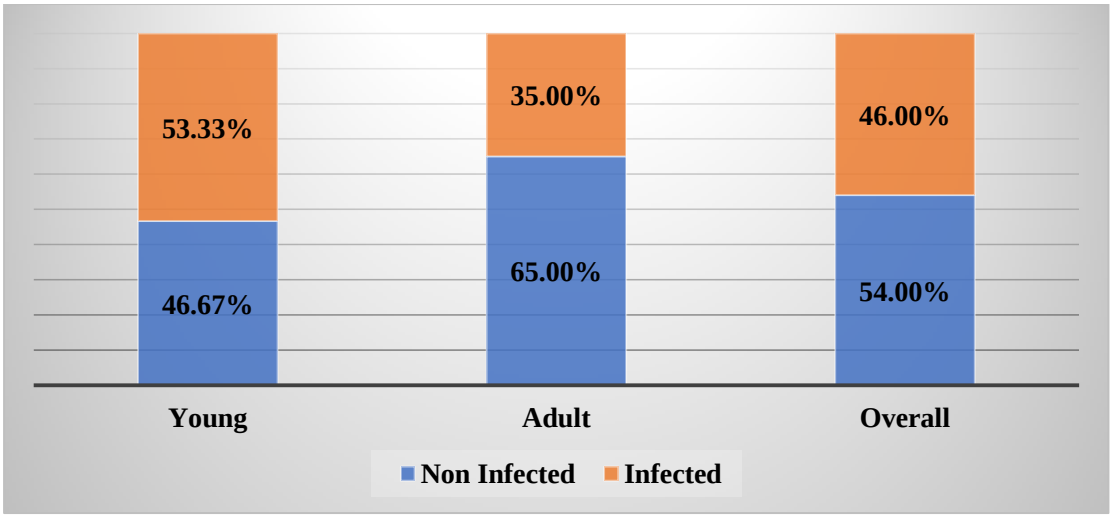

Figure 28. Host age wise distribution of *C. hepaticum* infection in *B. bengalensis* collected from fish market

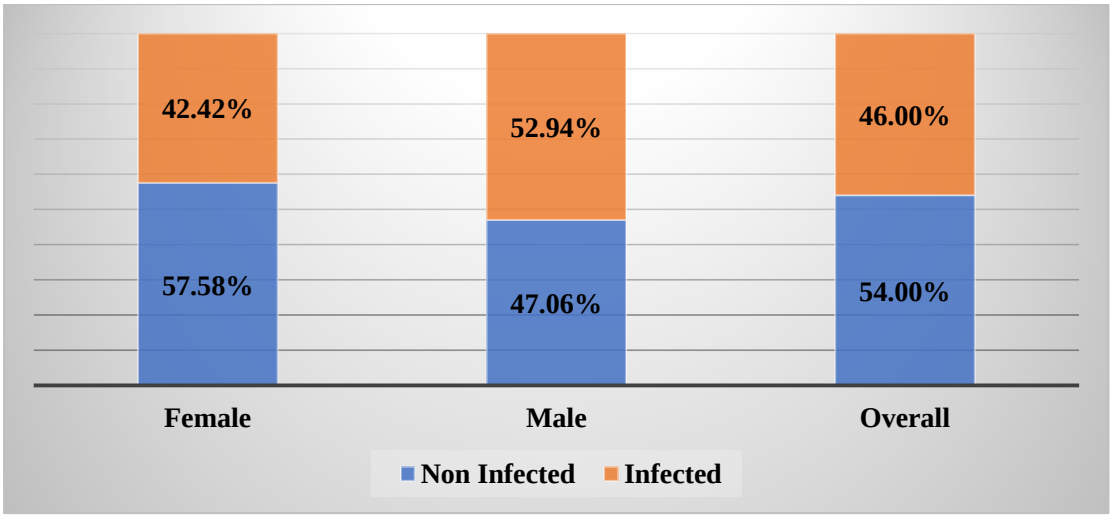

Figure 29. Host sex wise distribution of *C. hepatium* infection in *B. bengalensis* collected from fish market

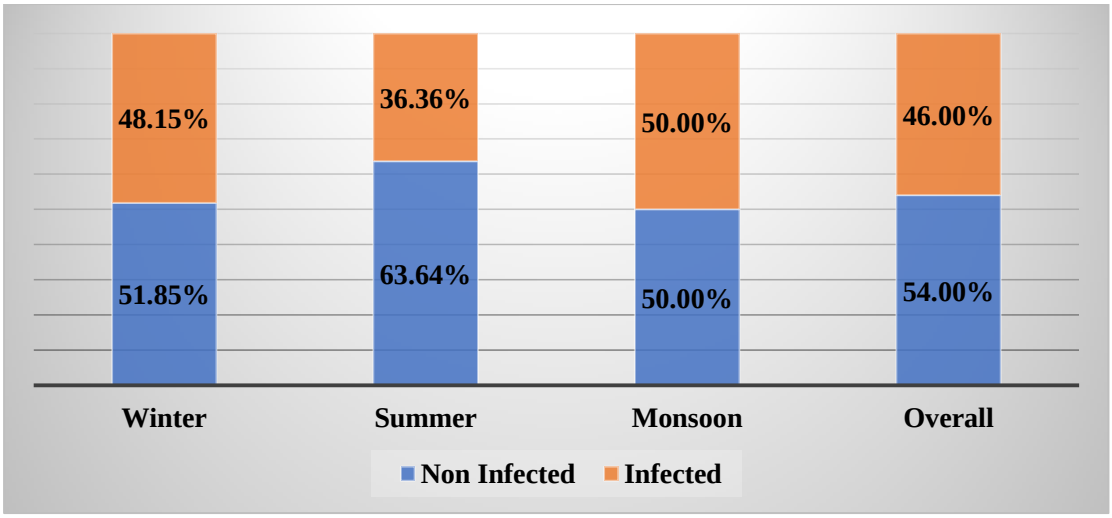

Figure 30. Season wise distribution of *C. hepaticum* infection in *B. bengalensis* collected from fish market

4.3.1.2.2.3 Analysis of *S. muris*

Out of total 50 rats, 14.00% (7) were found infected with *S. muris*. Percent infection was higher in monsoon (50.00%) followed by summer (9.09%) season. No infection was found in rats collected in winter season. Similarly, percent infection was higher in female (15.15%) and young rats (16.67%) indicating higher relative risk of disease transmission from young and female rats in monsoon season as compared to adult and male rats in winter and summer seasons (Figures 31-33). Total 351 numbers of parasites were recovered from seven rats. Prevalence of infection was more in young rats (63.25%) and female rats (65.24%). Prevalence of parasites was highest in monsoon season (94.59%) compared to summer (5.41%) season. The parasite intensity was high in adult rats and male rats in monsoon season, while the parasite index was high in young and male rats in monsoon season. Overall mean parasite intensity and parasite index were 50.14 and 7.02, respectively. Statistical analysis of the data revealed that season had significant (χ^2 = 17.53, P = 0.0002 and df = 2) effect on the prevalence of *S. muris* infection while host age and sex had no significant (P >0.05) effect (Table 28).

4.3.1.2.2.4 Analysis of *T. muris*

Out of total 50 rats, 26.00% (13) were found infected with *T. muris*. Percent infection was higher in monsoon (41.67%) followed by summer (27.27%) and winter (18.52%) seasons. Similarly, percent infection was higher in female (27.27%) and young rats (26.67%) indicating higher relative risk of disease transmission from young and female rats in monsoon season as compared to adult and male rats in winter and summer seasons (Figures 34-36). Total 70 numbers of parasites were recovered from 13 rats. Prevalence of infection was more in young rats (71.42%) and female rats (85.71%). Prevalence of parasites was high in winter season (57.14%) compared to monsoon (22.85%) and summer (20.00%) seasons.

Table 28. Risk factor analysis of *S. muris* infection in *B. bengalensis* collected from fish market

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	30	5(16.67)	1.67	222	63.25	44.40	7.40	0.44	0.51 (1)	0.35	7.76
	Adult	20	2(10.00)	1.00	129	36.75	64.50	6.45			0.15	6.42
Sex	Female	33	5(15.15)	1.29	229	65.24	45.80	6.94	0.11	0.74 (1)	0.27	5.95
	Male	17	2(11.76)	1.00	122	34.76	61.00	7.18			0.15	6.30
Season	Winter	27	0(0.00)	1.00	0	0.00	0.00	0.00	17.53	0.0002 (2)	0.02	48.65
	Summer	11	1(9.09)	9.09	19	5.41	19.00	1.73			0.30	159.86
	Monsoon	12	6(50.00)	50.00	332	94.59	55.33	27.67			1.70	460.57
Overall		**50**	**7(14.00)**	**-**	**351**	**-**	**50.14**	**7.02**	**-**	**-**	**-**	**-**

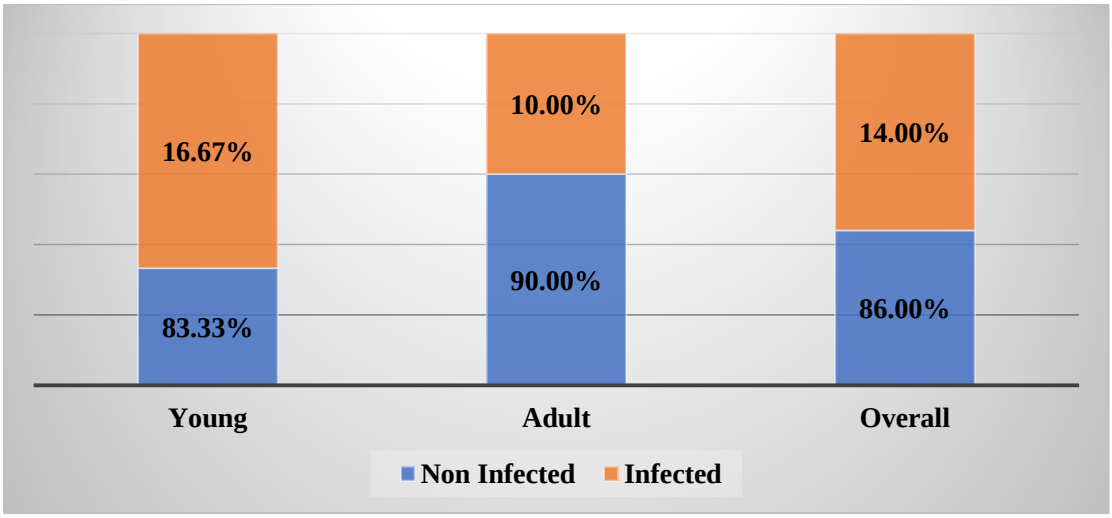

Figure 31. Host age wise distribution of *C. hepaticum* infection in *B. bengalensis* collected from fish market

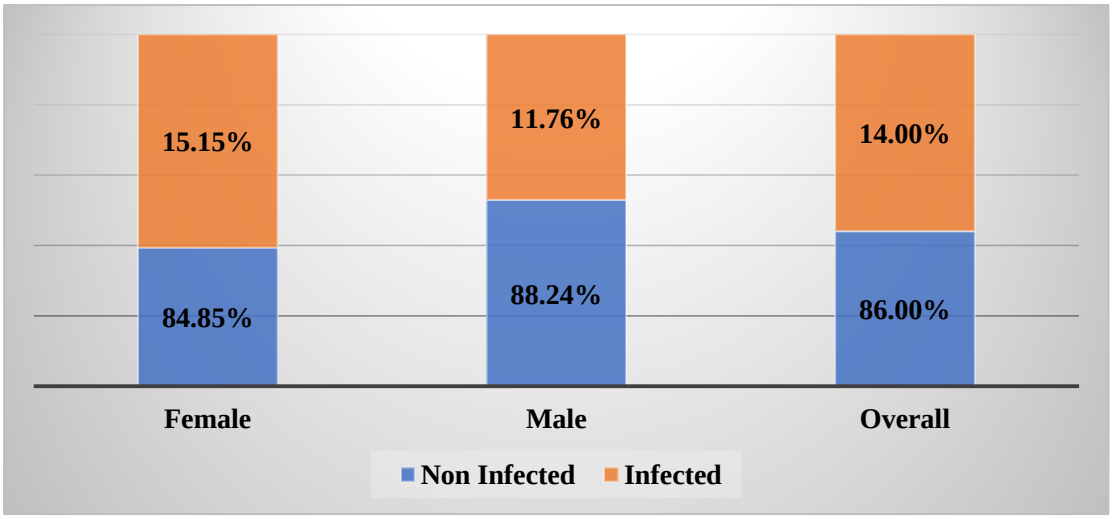

Figure 32. Host sex wise distribution of *C. hepaticum* infection in *B. bengalensis* collected from fish market

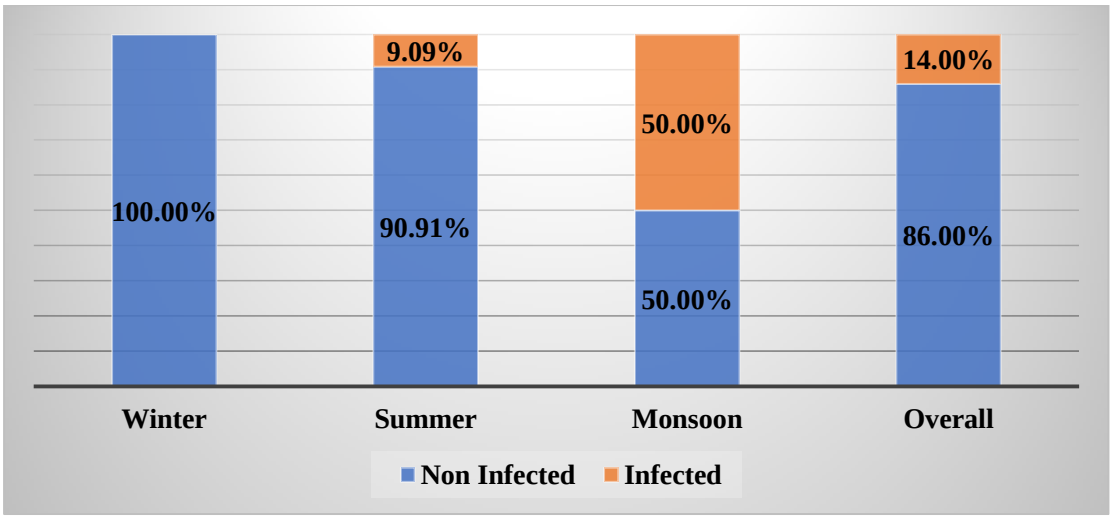

Figure 33. Season wise distribution of *C. hepaticum* infection in *B. bengalensis* collected from fish market

Table 29. Risk factor analysis of *T. muris* infection in *B. bengalensis* collected from fish market

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	30	8(26.67)	1.06	50	71.43	6.25	1.06	0.02	0.89 (1)	0.40	2.79
	Adult	20	5(25.00)	1.00	20	28.57	4.00	1.00			0.34	2.93
Sex	Female	33	9(27.27)	1.15	60	85.71	6.67	1.81	0.08	0.77 (1)	0.41	3.22
	Male	17	4(23.53)	1.00	10	14.29	2.50	0.59			0.29	3.36
Season	Winter	27	5(18.52)	1.00	40	57.14	8.00	1.48	2.33	0.31 (2)	0.32	3.06
	Summer	11	3(27.27)	1.47	14	20.00	4.67	1.27			0.42	5.13
	Monsoon	12	5(41.67)	2.25	16	22.86	3.20	1.33			0.79	6.34
Overall		**50**	**13(26.00)**	**-**	**70**	**-**	**5.38**	**1.40**	**-**	**-**	**-**	**-**

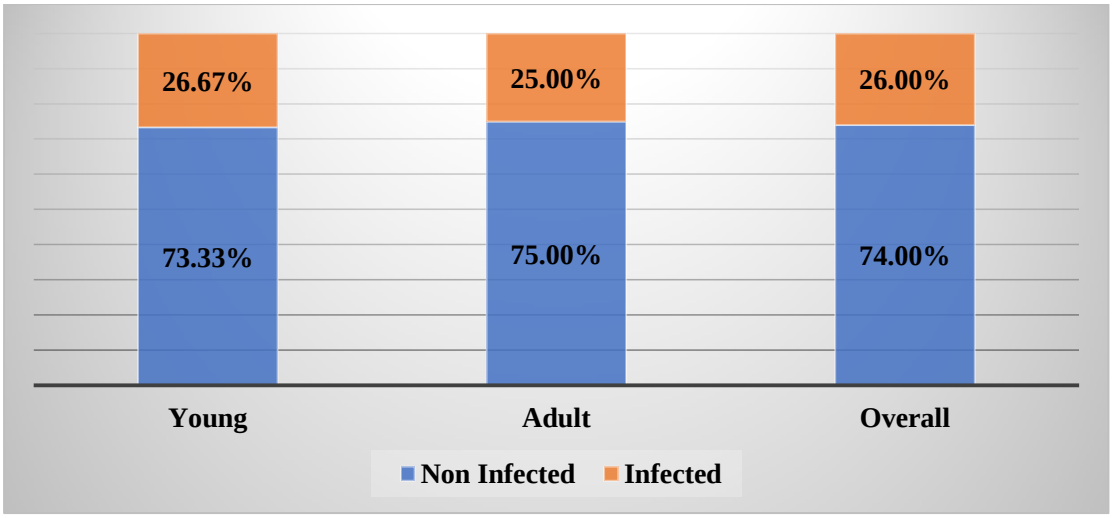

Figure 34. Host age wise distribution of *T. muris* infection in *B. bengalensis* collected from fish market

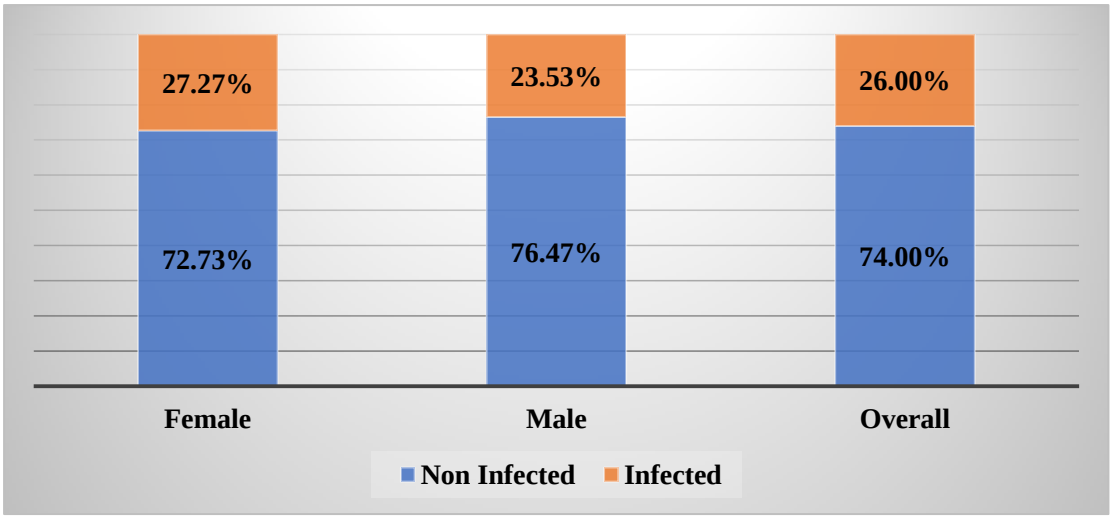

Figure 35. Host sex wise distribution of *T. muris* infection in *B. bengalensis* collected from fish market

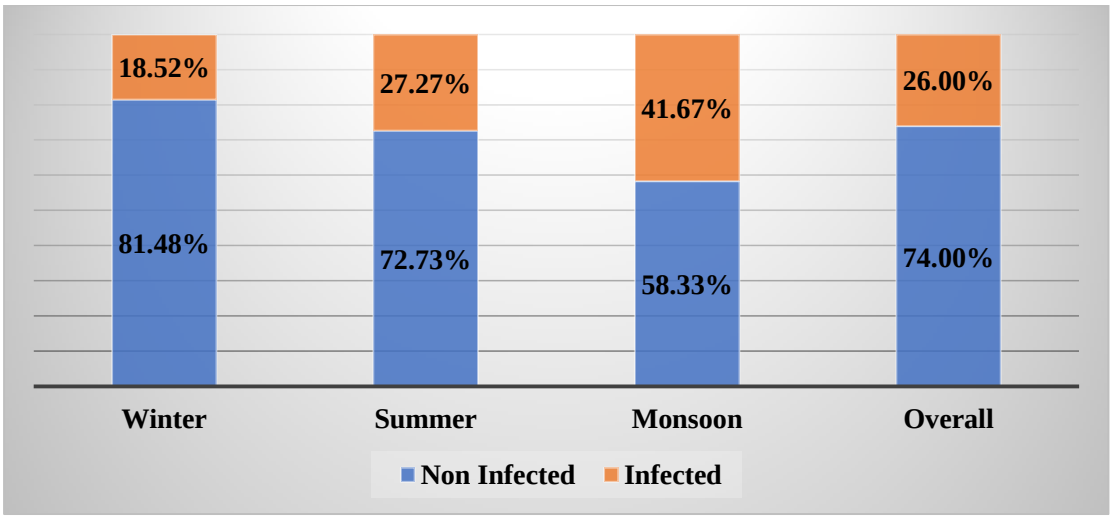

Figure 36. Season wise distribution of *T. muris* infection in *B. bengalensis* collected from fish market

The parasite intensity and parasite index were also high in young rats and female rats in winter season. Overall mean parasite intensity and parasite index were 5.38 and 1.40, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of *T. muris* infection (Table 29).

4.3.1.2.2.5 Analysis of *H. spumosa*

Out of total 50 rats, 20.00% (10) were found infected with *H. spumosa*. Percent infection was higher in monsoon (33.33%) followed by summer (27.27%) and winter (11.11%) seasons. Similarly, percent infection was higher in female (27.27%) and adult rats (25.00%) indicating higher relative risk of disease transmission from adult and female rats in monsoon season as compared to young and male rats in winter and summer seasons (Figures 37-39). Total 135 numbers of parasites were recovered from 10 rats. Prevalence of infection was more in young rats (60.00%) and female rats (93.33%). Prevalence of parasites was high in summer season (38.51%) compared to winter (37.77%) and monsoon (23.70%) seasons. The parasite intensity was high in young rats and female rats in summer season. Parasite index was same in young and adult rats. It was high in female rats and in summer season. Overall mean parasite intensity and parasite index were 13.50 and 2.70, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of *H. spumosa* infection (Table 30).

4.3.1.2.2.6 Overall analysis of nematodes

Out of total 50 rats, 72.00% (36) were found infected with five species of nematodes. Percent infection was higher in monsoon (75.00%) followed by winter (74.07%) and summer (63.64%) seasons. Similarly, percent infection was higher in female (72.73%) and young rats (73.33%) indicating higher relative risk of disease transmission from young and female rats in monsoon season as compared to adult and male rats in winter and summer seasons (Figures 40-42). Total 1469 numbers of parasites were recovered from 36 rats. Prevalence of infection was higher in young rats (67.60%) and female rats (62.08%). Prevalence of parasites was higher in winter season (52.08%) compared to monsoon (28.11%) and summer (19.81%) seasons. The parasite intensity was high in young rats and male rats in monsoon season. The parasite index and parasite index were high in adult rats and male rats in winter season. Overall mean parasite intensity and parasite index were 40.80 and 29.38, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of overall nematode infection (Table 31).

4.3.1.2.3 Overall analysis of endoparasites found in rats collected from fish market

Overall, out of total 50 rats, 86.00% (43) were found infected with two species of cestodes and five species of nematodes. Percent infection was higher in winter (100.00%) followed by monsoon (75.00%) and summer (63.64%) seasons. Similarly, percent infection was higher in male (94.12%) and young rats (86.67%) indicating higher relative risk of

Table 30. Risk factor analysis of *H. spumosa* infection in *B. bengalensis* collected from fish market

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	30	5(16.67)	1.00	81	60.00	16.20	2.70	0.52	0.47 (1)	0.32	3.10
	Adult	20	5(25.00)	1.50	54	40.00	10.80	2.70			0.49	4.51
Sex	Female	33	9(27.27)	4.64	126	93.33	14.00	3.82	3.21	0.07 (1)	0.63	33.62
	Male	17	1(5.88)	1.00	9	6.67	9.00	0.53			0.06	14.71
Season	Winter	27	3(11.11)	1.00	51	37.78	17.00	1.89	3.03	0.22 (2)	0.22	4.52
	Summer	11	3(27.27)	2.45	52	38.52	17.33	4.73			0.58	10.34
	Monsoon	12	4(33.33)	3.00	32	23.70	8.00	2.67			0.79	11.38
Overall		**50**	**10(20.00)**	**-**	**135**	**-**	**13.50**	**2.70**	**-**	**-**	**-**	**-**

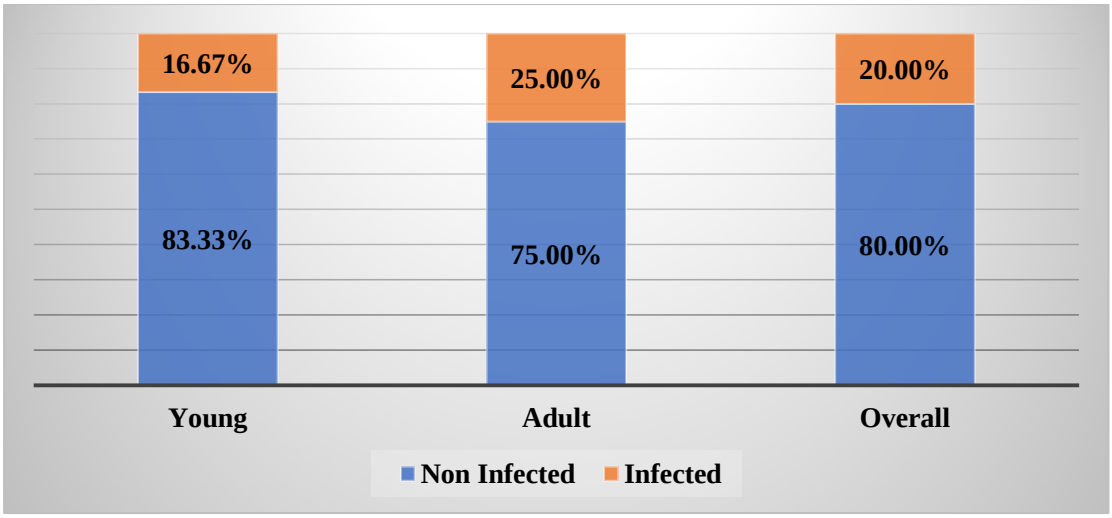

Figure 37. Host age wise distribution of *H. spumosa* infection in *B. bengalensis* collected from fish market

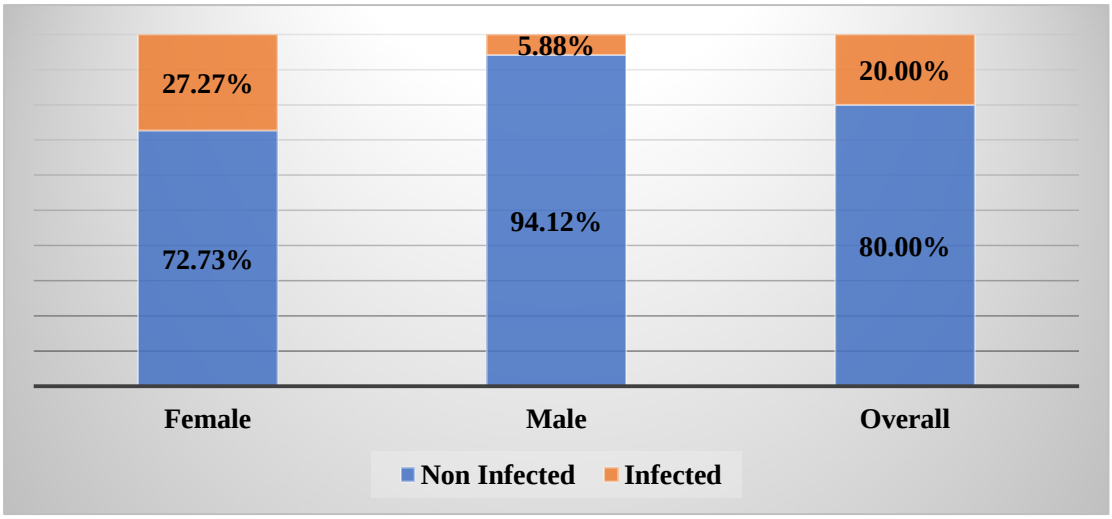

Figure 38. Host sex wise distribution of *H. spumosa* infection in *B. bengalensis* collected from fish market

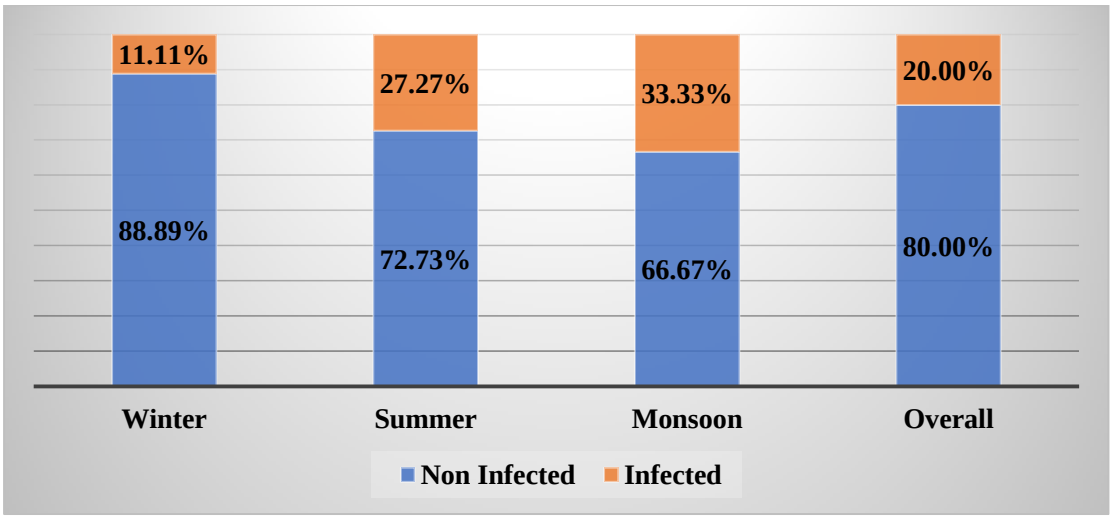

Figure 39. Season wise distribution of *H. spumosa* infection in *B. bengalensis* collected from fish market

Table 31. Risk factor analysis of nematode infection in *B. bengalensis* collected from fish market

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	30	22(73.33)	1.05	993	67.60	45.14	33.10	0.07	0.80 (1)	0.73	1.50
	Adult	20	14(70.00)	1.00	476	32.40	34.00	23.80			0.66	1.50
Sex	Female	33	24(72.73)	1.03	912	62.08	38.00	27.64	0.02	0.87 (1)	0.71	1.49
	Male	17	12(70.59)	1.00	557	37.92	46.42	32.76			0.64	1.54
Season	Winter	27	20(74.07)	1.16	765	52.08	38.25	28.33	0.49	0.78 (2)	0.70	1.91
	Summer	11	7(63.64)	1.00	291	19.81	41.57	26.45			0.53	1.88
	Monsoon	12	9(75.00)	1.18	413	28.11	45.89	34.42			0.67	2.04
Overall		**50**	**36(72.00)**	-	**1469**	-	**40.80**	**29.38**	-	-	-	-

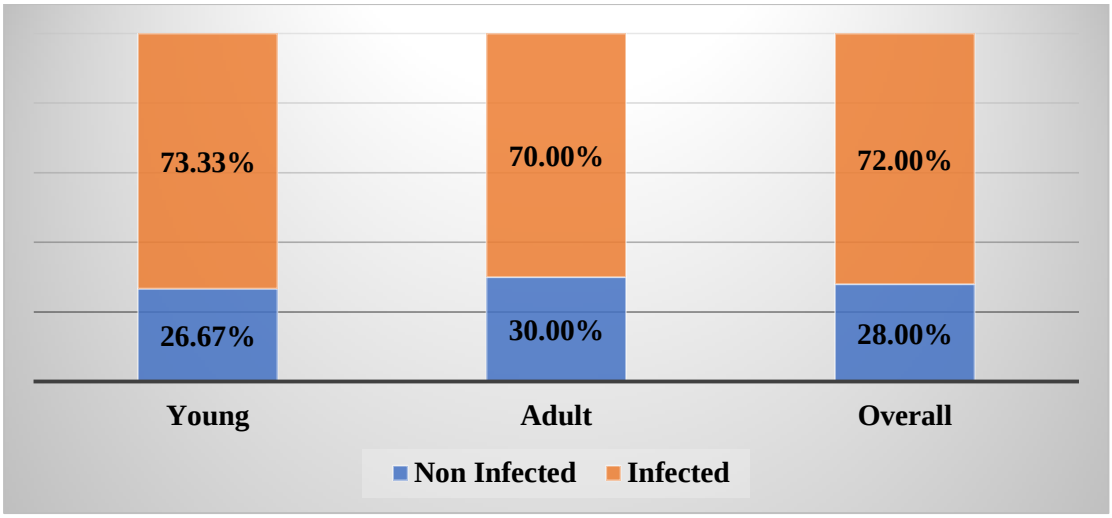

Figure 40. Host age wise distribution of nematode infection in *B. bengalensis* collected from fish market

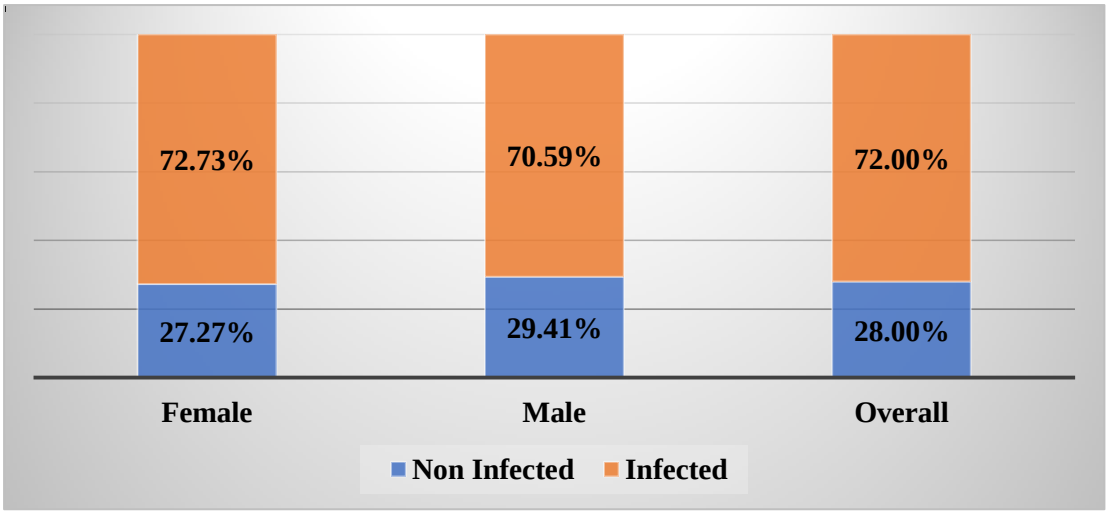

Figure 41. Host sex wise distribution of nematode infection in *B. bengalensis* collected from fish market

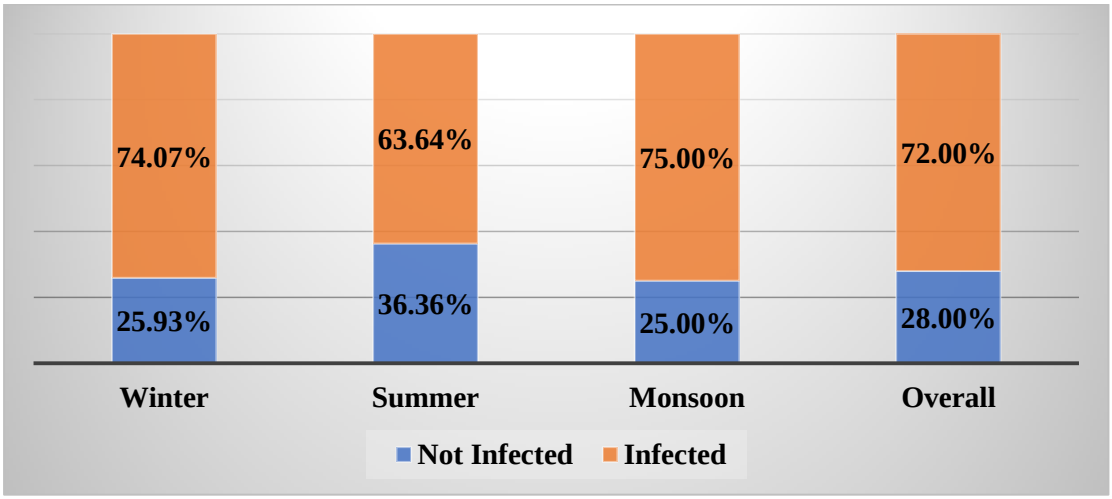

Figure 42. Season wise distribution of nematode infection in *B. bengalensis* collected from fish market

Table 32. Risk factor analysis of overall endoparasitic infection in *B. bengalensis* collected from fish market

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	30	26(86.67)	1.02	1056	66.33	40.61	35.20	0.03	0.87 (1)	0.81	1.28
	Adult	20	17(85.00)	1.00	536	33.67	31.53	26.80			0.77	1.29
Sex	Female	33	27(81.82)	1.00	987	62.00	36.56	29.91	1.41	0.23 (1)	0.79	1.26
	Male	17	16(94.12)	1.15	605	38.00	37.81	35.59			0.94	1.41
Season	Winter	27	27(100.00)	1.57	879	55.21	32.56	32.55	10.17	0.0062 (2)	1.00	2.45
	Summer	11	7(63.64)	1.00	297	18.65	42.43	27.00			0.53	1.88
	Monsoon	12	9(75.00)	1.18	416	26.13	46.22	34.67			0.67	2.04
Overall		**50**	**43(86.00)**	**-**	**1592**	**-**	**37.02**	**31.84**	**-**	**-**	**-**	**-**

disease transmission from young and male rats in winter season as compared to adult and female rats in monsoon and summer seasons. Total 1592 numbers of parasites (except *C. hepaticum*) were recovered from 43 rats. Prevalence of infection was more in young rats (66.33%) and female rats (62.00%). Prevalence of parasites was high in winter season (55.21%) compared to monsoon (26.13%) and summer (18.65%) seasons. The parasite intensity and parasite index were high in young and male rats in monsoon season. Overall mean parasite intensity and parasite index were 37.02 and 31.84, respectively. Statistical analysis of the data revealed that season had significant (χ^2 = 10.17, P = 0.0062 and df = 2) effect on the prevalence of overall endoparasitic infection while host age and sex had no significant (P >0.05) effect (Table 32).

4.3.2 Analysis of rats collected from railway station

4.3.2.1 Overall analysis of endoparasites found in rats collected from railway station

Out of total 50 rats collected from railway station and examined for the presence of endoparasites, 74.00% (37 rats) were found infected with one or more species of endoparasites. Their season, host sex and age wise distribution is presented herewith.

4.3.2.1 Season wise distribution of endoparasites

In winter season, 91.67% (11) rats collected were found infected with endoparasites. While 84.62% (11) and 60.00% (15) rats were found infected with endoparasites in summer and monsoon seasons, respectively (Table 33). This predicted higher rate of endoparasitic infestation and relative risk of disease transmission in winter season followed by summer and monsoon seasons (Figure 43).

Table 33. Season wise distribution of endoparasitic infection in *B. bengalensis* collected from railway station

Status/season	Winter	Summer	Monsoon	Overall
Infected	11(91.67)	11(84.62)	15(60.00)	37(74.00)
Non-infected	1(8.33)	2(15.38)	10(40.00)	13(26.00)
Total	**12(100.00)**	**13(100.00)**	**25(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

4.3.2.2 Host sex wise distribution of endoparasites

Out of 27 female and 23 male rats examined, 65.22% male (15) and 81.48% female (22) rats were found infected with endoparasites. This predicted higher rate of endoparasitic infestation in females than that in males (Table 34) indicating relatively higher risk of disease transmission from female rats as compared to male rats (Figure 44).

Table 34. Host sex wise distribution of endoparasitic infection in *B. bengalensis* collected from railway station

Status/sex	Female	Male	Overall
Infected	22(81.48)	15(65.22)	37(74.00)
Non-infected	5(18.52)	8(34.78)	13(26.00)
Total	**27(100.00)**	**23(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

4.3.2.3 Host age wise distribution of endoparasites

Out of 22 young and 28 adult rats examined, 86.36% young (19) and 64.28% adult (18) rats were found infected with endoparasites. This predicted slightly higher rate of endoparasitic infestation in young individuals than that in adults (Table 35) indicating relatively higher risk of disease transmission from young rats as compared to adults (Figure 45).

Table 35. Host age wise distribution of endoparasitic infection in *B. bengalensis* collected from railway station

Status/age	Young	Adult	Overall
Infected	19(86.36)	18(64.28)	37(74.00)
Non-infected	3(13.63)	10(35.71)	13(26.00)
Total	**22(100.00)**	**28(100.00)**	**50(100.00)**

Figures in the parentheses are percentages from total

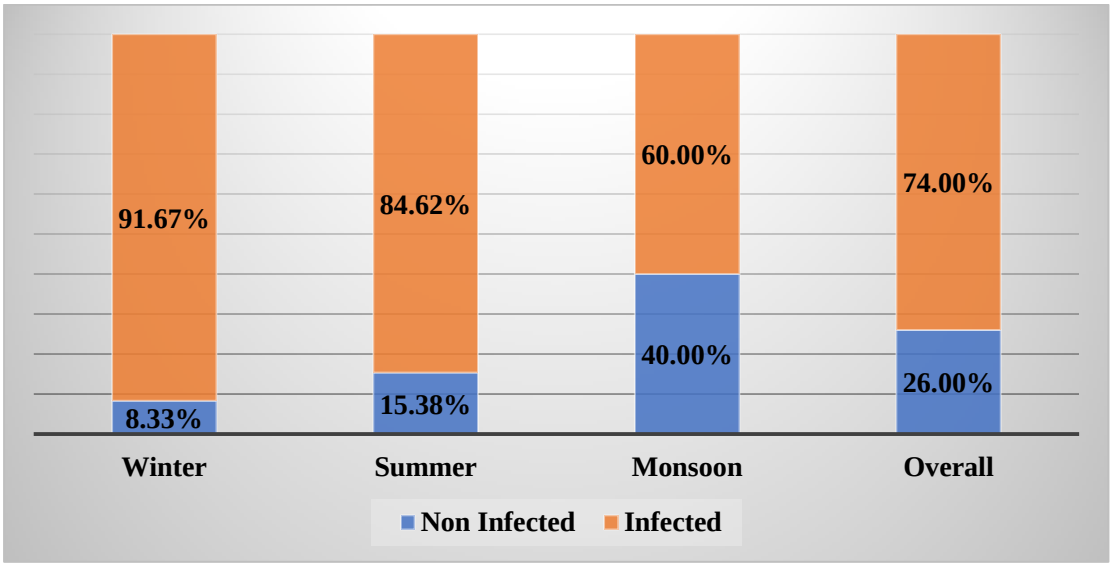

Figure 43. Season wise distribution of infection in *B. bengalensis* collected from railway station

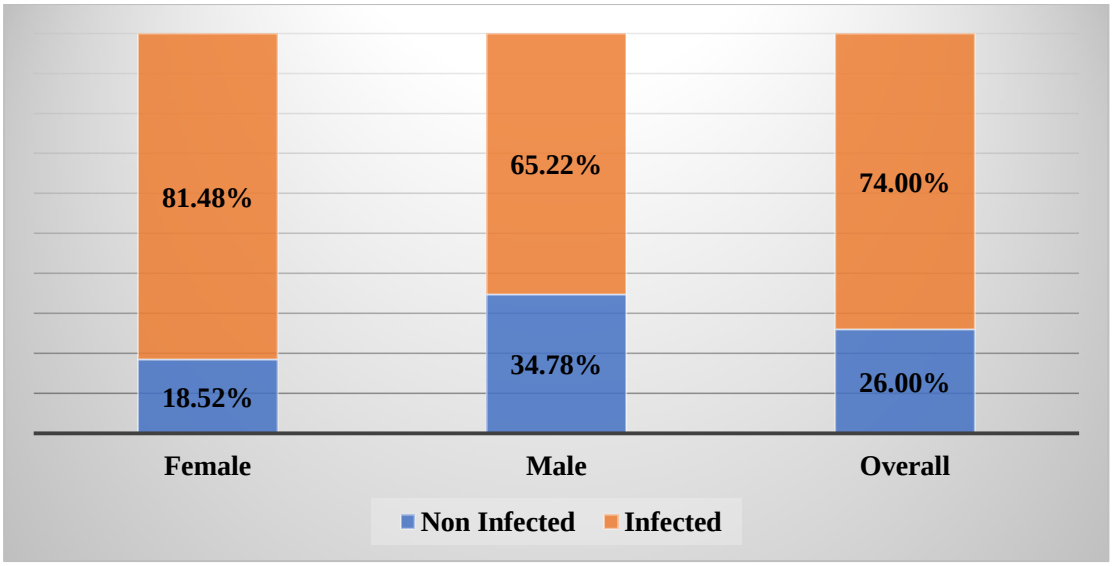

Figure 44. Host sex wise distribution of infection in *B. bengalensis* collected from railway station

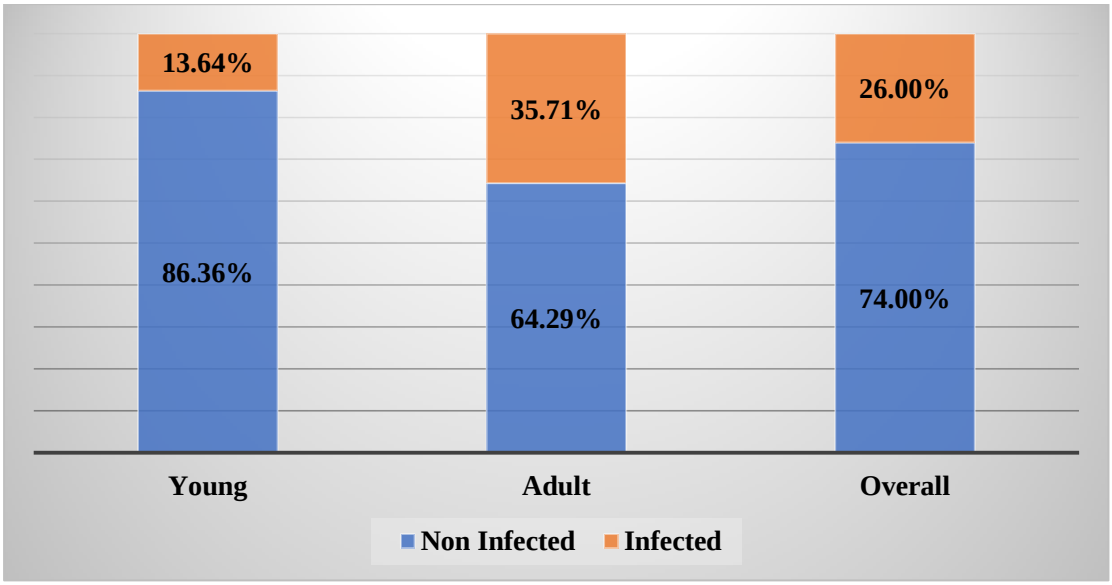

Figure 45. Host age wise distribution of infection in *B. bengalensis* collected from railway station

4.3.2.2 Individual analysis of endoparasites found in rats collected from railway station

Out of total 50 rats examined, 74.00% (37) were found infected with same seven species of endoparasites i.e., two species of cestodes (*H. nana* and *C. fasciolaris*) and five species of nematodes (*N. brasiliensis, C. hepaticum, S muris, T. muris* and *S. ratti*). The site of infection of these parasites was same as that found in rats collected from fish market. Rats were found infected with one or more number of parasite species simultaneously indicating concurrent infection (Table 36). Prevalence, intensity and risk factor analysis of all the endoparasites is presented herewith individually.

Table 36. Overall infection of endoparasites in *B. bengalensis* collected from railway station

Class of endoparasites	Name of endoparasite	Host infected (n = 50)	
		Number	Percentage
Cestoda	*H. nana*	12	24.00
	C. fasciolaris	10	20.00
Nematoda	*N. brasiliensis*	19	38.00
	C. hepaticum	23	46.00
	S. muris	6	12.00
	T. muris	5	10.00
	H. spumosa	15	30.00
Overall		**90***	**-**

*Concurrent infection in rats with one or more endoparasites

4.3.2.2.1 Analysis of cestode parasites

4.3.2.2.1.1 Analysis of *H. nana*

Out of total 50 rats, 24.00% (12) were found infected with *H. nana*. Percent infection was higher in monsoon (28.00%) followed by winter (25.00%) and summer (15.38%) seasons. Similarly, percent infection was higher in female (29.63%) and adult rats (25.00%) indicating higher relative risk of disease transmission from adult and female rats in monsoon season as compared to young and male rats in winter and summer seasons (Figures 46-48). Total 45 numbers of parasites were recovered from 12 rats. Prevalence of infection was more in adult rats (51.11%) and female rats (71.11%). Prevalence of parasites was high in monsoon season (51.11%) compared to summer (31.11%) and winter (17.78%) seasons. The parasite intensity and parasite index were high in young rats and female rats in summer season. Overall mean parasite intensity and parasite index were 3.75 and 0.90, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of *H. nana* infection (Table 37).

4.3.2.2.1.2 Analysis of *C. fasciolaris*

Out of total 50 rats, 20.00% (10) were found infected with *C. fasciolaris*. Percent infection was higher in winter (25.00%) followed by summer (23.07%) and monsoon (16.00%) seasons. Similarly, percent infection was higher in female (22.22%) and adult rats (21.43%) indicating higher relative risk of disease transmission from adult and female rats in winter season as compared to young and male rats in summer and monsoon seasons (Figures 49-51). Total 15 numbers of parasites were recovered from 10 rats. Prevalence of infection was more in adult rats (60.00%) and male rats (53.33%). Prevalence of parasites was high in monsoon season (40.00%) compared to summer (33.33%) and winter (26.67%) seasons. The parasite intensity was similar in young and adult rats, but it was high in female rat's summer

Table 37. Risk factor analysis of *H. nana* infection in *B. bengalensis* collected from railway station

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	22	5(22.73)	1.00	22	48.89	4.44	1.00	0.03	0.85 (1)	0.34	2.97
	Adult	28	7(25.00)	1.10	23	51.11	3.28	0.82			0.40	3.00
Sex	Female	27	8(29.63)	1.70	32	71.11	4.00	1.18	1.02	0.31 (1)	0.55	4.61
	Male	23	4(17.39)	1.00	13	28.89	3.25	0.56			0.28	3.52
Season	Winter	12	3(25.00)	1.62	8	17.78	2.67	0.67	0.75	0.68 (2)	0.32	8.11
	Summer	13	2(15.38)	1.00	14	31.11	7.00	1.08			0.16	6.06
	Monsoon	25	7(28.00)	1.82	23	51.11	3.28	0.92			0.44	7.54
Overall		**50**	**12(24.00)**	**-**	**45**	**-**	**3.75**	**0.90**	**-**	**-**	**-**	**-**

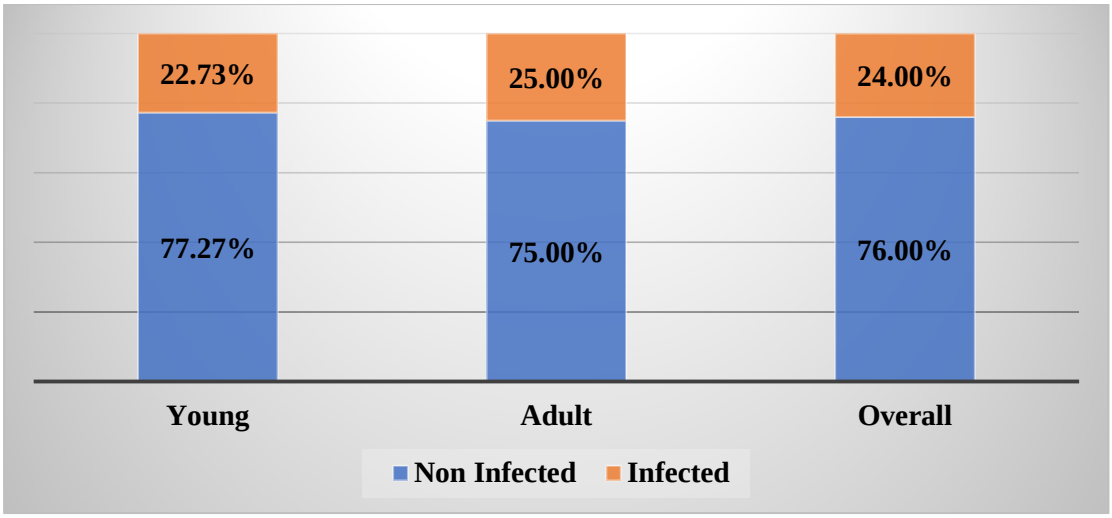

Figure 46. Host age wise distribution of *H. nana* infection in *B. bengalensis* collected from railway station

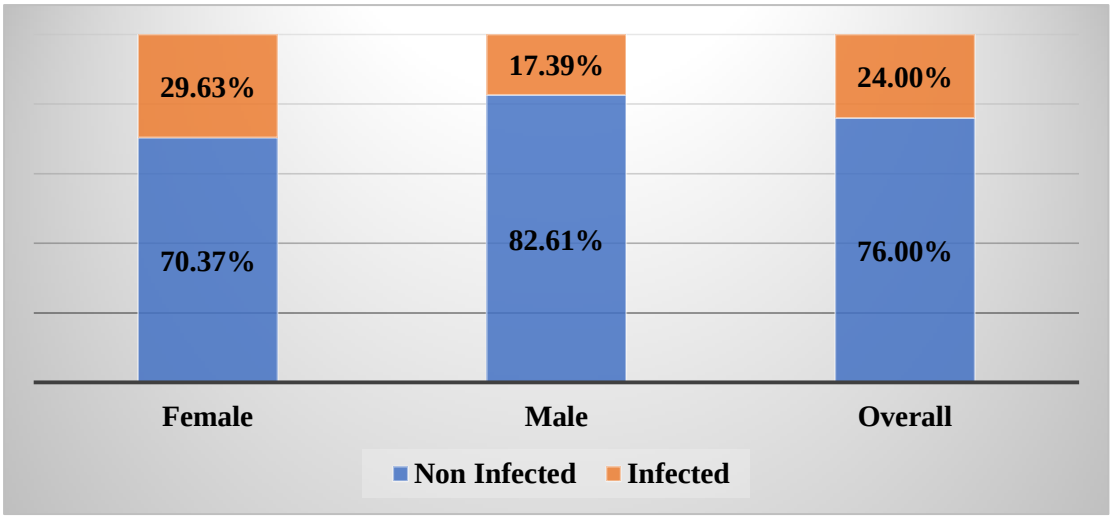

Figure 47. Host sex wise distribution of *H. nana* infection in *B. bengalensis* collected from railway station

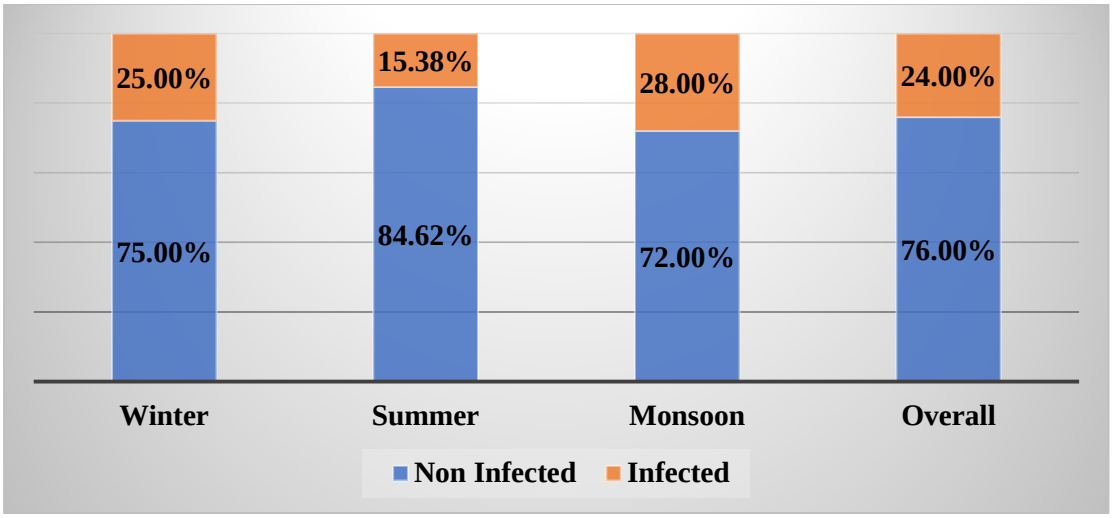

Figure 48. Season wise distribution of *H. nana* infection in *B. bengalensis* collected from railway station

Table 38. Risk factor analysis of *C. fasciolaris* infection in *B. bengalensis* collected from railway station

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	22	4(18.18)	1.00	6	40.00	1.50	0.27	0.08	0.77 (1)	0.28	3.50
	Adult	28	6(21.43)	1.18	9	60.00	1.50	0.32			0.37	3.66
Sex	Female	27	6(22.22)	1.28	7	46.67	1.17	0.26	0.18	0.67 (1)	0.41	3.98
	Male	23	4(17.39)	1.00	8	53.33	2.00	0.34			0.28	3.52
Season	Winter	12	3(25.00)	1.56	4	26.67	1.33	0.33	0.51	0.77 (2)	0.41	5.90
	Summer	13	3(23.08)	1.44	5	33.33	1.67	0.38			0.37	5.50
	Monsoon	25	4(16.00)	1.00	6	40.00	1.50	0.24			0.28	3.56
Overall		**50**	**10(20.00)**	**-**	**15**	**-**	**1.50**	**0.30**	**-**	**-**	**-**	**-**

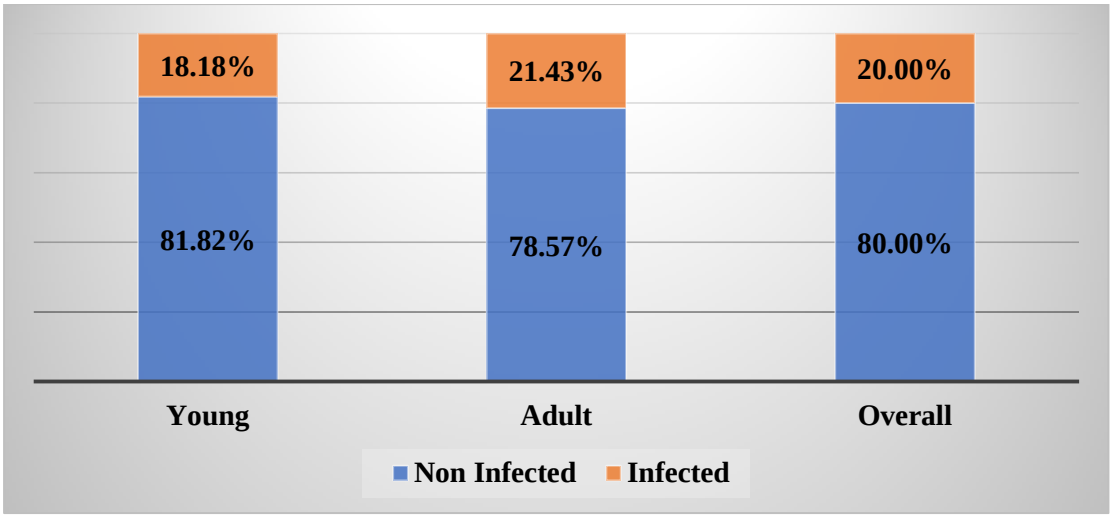

Figure 49. Host age wise distribution of *C. fascioloaris* infection in *B. bengalensis* collected from railway station

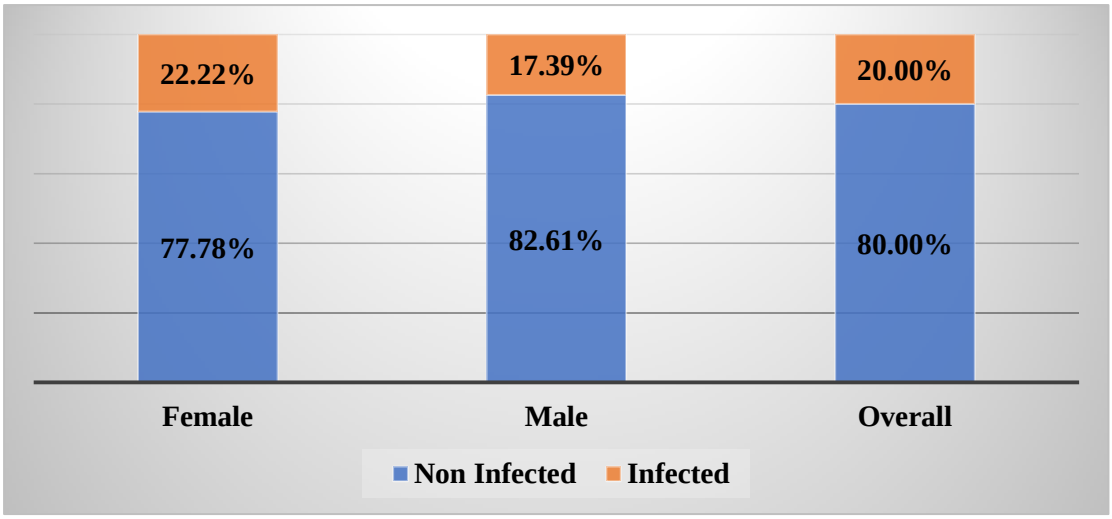

Figure 50. Host sex wise distribution of *C. fascioloaris* infection in *B. bengalensis* collected from railway station

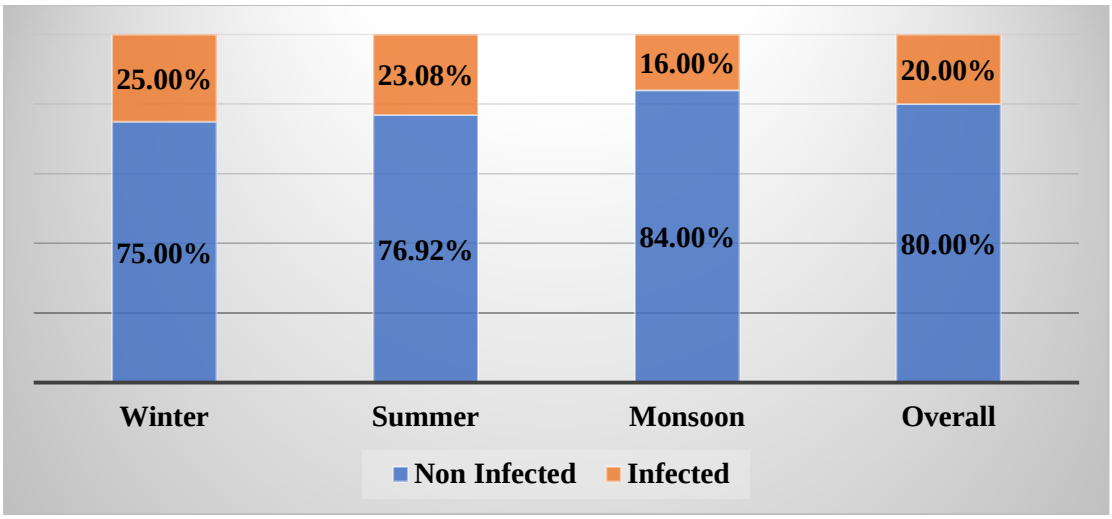

Figure 51. Season wise distribution of *C. fascioloaris* infection in *B. bengalensis* collected from railway station

season. The parasite index was high in adult rats and male rats in summer season. Overall mean parasite intensity and parasite index were 1.50 and 0.30, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of *C. fasciolaris* infection (Table 38).

4.3.2.2.1.3 Overall analysis of cestode parasites

In overall, out of total 50 rats, 38.00% (19) were found infected with two species of cestodes. Percent infection was higher in winter (41.67%) followed by summer (38.46%) and monsoon (36.00%) seasons. Similarly, percent infection was higher in female (44.44%) and adult rats (39.28%) indicating higher relative risk of disease transmission from adult and female rats in winter season as compared to young and male rats in summer and monsoon seasons (Figures 52-54). Total 60 numbers of parasites were recovered from 19 rats. Prevalence of infection was more in young rats (46.67%) and female rats (65.00%). Prevalence of parasites was high in monsoon season (48.33%) compared to summer (31.66%) and winter (20.00%) seasons. The parasite intensity and parasite index were high in young and female rats in summer season. Overall mean parasite intensity and parasite index were 3.15 and 1.20 respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of *overall cestode* infection (Table 39).

4.3.2.2.2 Analysis of nematode parasites

4.3.2.2.2.1 Analysis of *N. brasiliensis*

Out of total 50 rats, 38.00% (19) were found infected with *N. brasiliensis*. Percent infection was higher in winter (75.00%) followed by summer (38.46%) and monsoon (20.00%) seasons. Similarly, percent infection was higher in female (44.44%) and young rats (40.90%) indicating higher relative risk of disease transmission from young and female rats in winter season as compared to adult and male rats in summer and monsoon seasons (Figures 55-57). Total 526 numbers of parasites were recovered from 19 rats. Prevalence of infection was more in young rats (59.31%) and female rats (77.95%). Prevalence of parasites was high in monsoon season (65.40%) compared to summer (20.72%) and winter (13.88%) seasons. The parasite intensity and parasite index were high in young and female rats in monsoon season. Overall mean parasite intensity and parasite index were 27.68 and 10.52, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of *N. brasiliensis* infection (Table 40).

4.3.2.2.2.2 Analysis of *C. hepaticum*

Out of total 50 rats, liver of 46.00% (23) was found infected with *C. hepaticum.* Percent infection was higher in summer season (61.54%) followed by winter (58.33%) and monsoon (32.00%) seasons. Similarly, percent infection was higher in female (51.85%) and young rats (50.00%) indicating higher relative risk of disease transmission from young and

Table 39. Risk factor analysis of cestode infection in *B. bengalensis* collected from railway station

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	22	8(36.36)	1.00	28	46.67	3.50	1.27	0.04	0.83 (1)	0.45	2.18
	Adult	28	11(39.28)	1.08	22	36.67	2.00	0.78			0.52	2.21
Sex	Female	27	12(44.44)	1.46	39	65.00	3.25	1.44	1.03	0.31 (1)	0.69	3.08
	Male	23	7(30.43)	1.00	21	35.00	3.00	0.91			0.41	2.39
Season	Winter	12	5(41.67)	1.16	12	20.00	2.40	1.00	0.11	0.94 (2)	0.49	2.70
	Summer	13	5(38.46)	1.06	19	31.67	3.80	1.46			0.45	2.53
	Monsoon	25	9(36.00)	1.00	29	48.33	3.22	1.16			0.47	2.09
Overall		**50**	**19(38.00)**	**-**	**60**	**-**	**3.15**	**1.20**	**-**	**-**	**-**	**-**

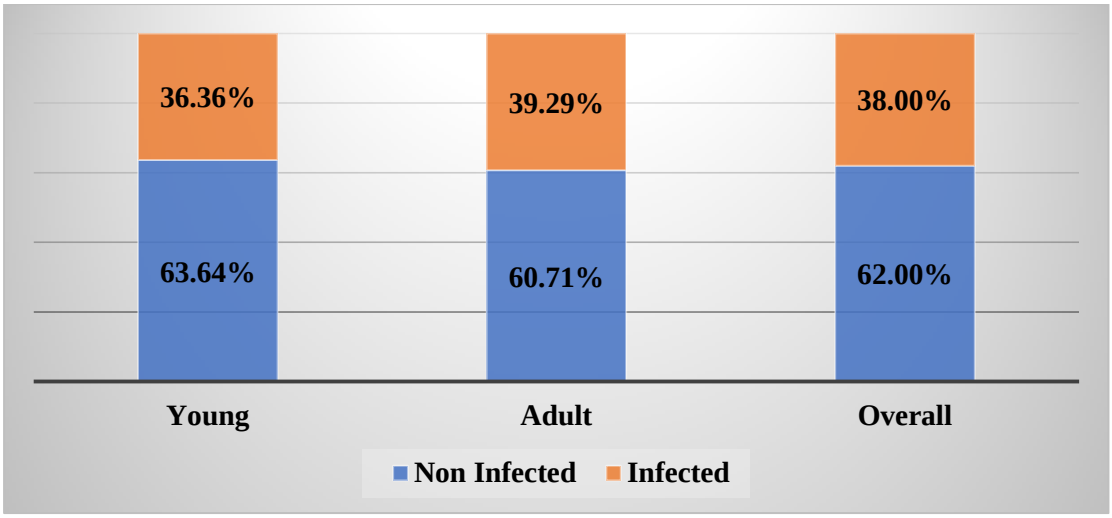

Figure 52. Host age wise distribution of cestode infection in *B. bengalensis* collected from railway station

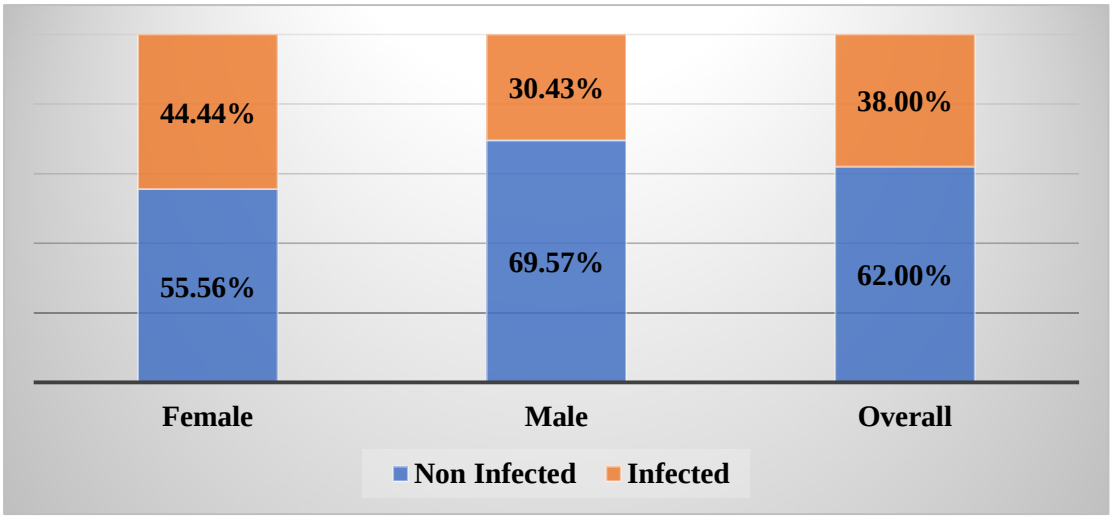

Figure 53. Host sex wise distribution of cestode infection in *B. bengalensis* collected from railway station

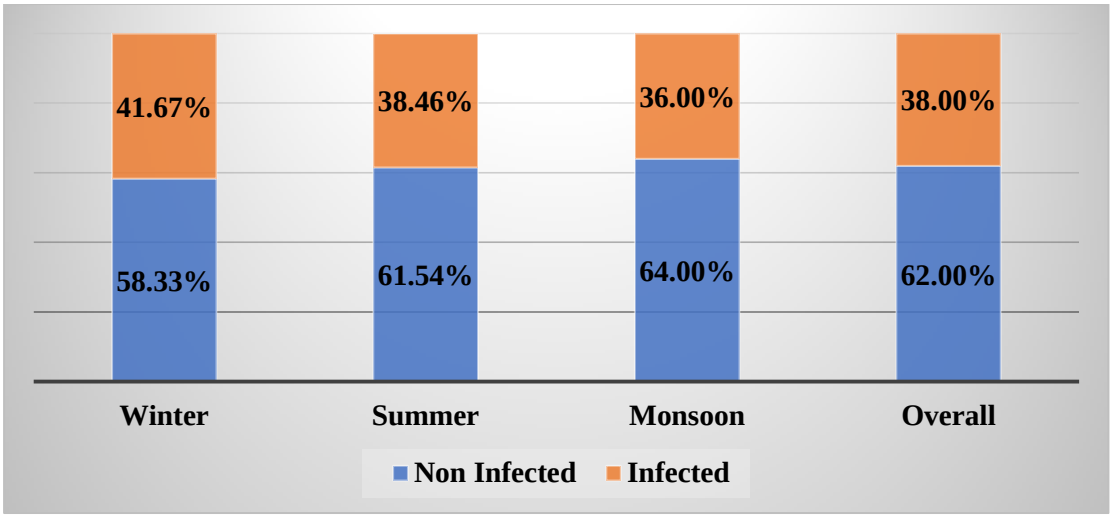

Figure 54. Season wise distribution of cestode infection in *B. bengalensis* collected from railway station

Table 40. Risk factor analysis of *N. brasiliensis* infection in *B. bengalensis* collected from railway station

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	22	9(40.90)	1.14	312	59.31	34.67	14.18	0.14	0.71 (1)	0.56	2.32
	Adult	28	10(35.71)	1.00	214	40.68	21.40	7.64			0.49	2.02
Sex	Female	27	12(44.44)	1.46	410	77.95	34.17	14.64	1.03	0.31 (1)	0.69	3.08
	Male	23	7(30.43)	1.00	116	22.05	16.57	5.27			0.41	2.39
Season	Winter	12	9(75.00)	3.75	73	13.88	8.11	6.08	0.11	0.94 (2)	1.60	8.76
	Summer	13	5(38.46)	1.92	109	20.72	21.80	8.38			0.67	5.45
	Monsoon	25	5(20.00)	1.00	344	65.40	68.80	13.76			0.33	3.03
Overall		**50**	**19(38.00)**	**-**	**526**	**-**	**27.68**	**10.52**	**-**	**-**	**-**	**-**

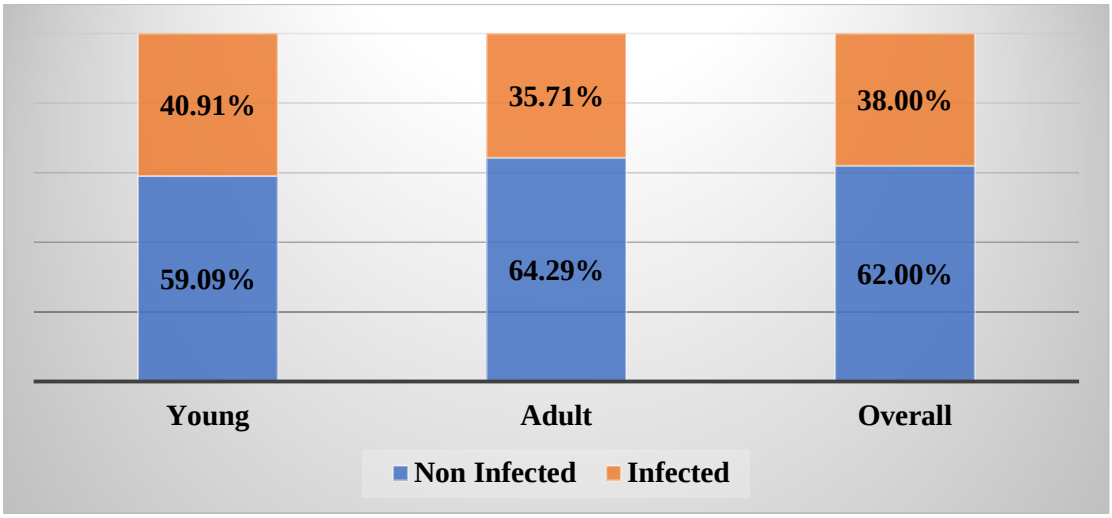

Figure 55. Host age wise distribution of *N. brasiliensis* infection in *B. bengalensis* collected from railway station

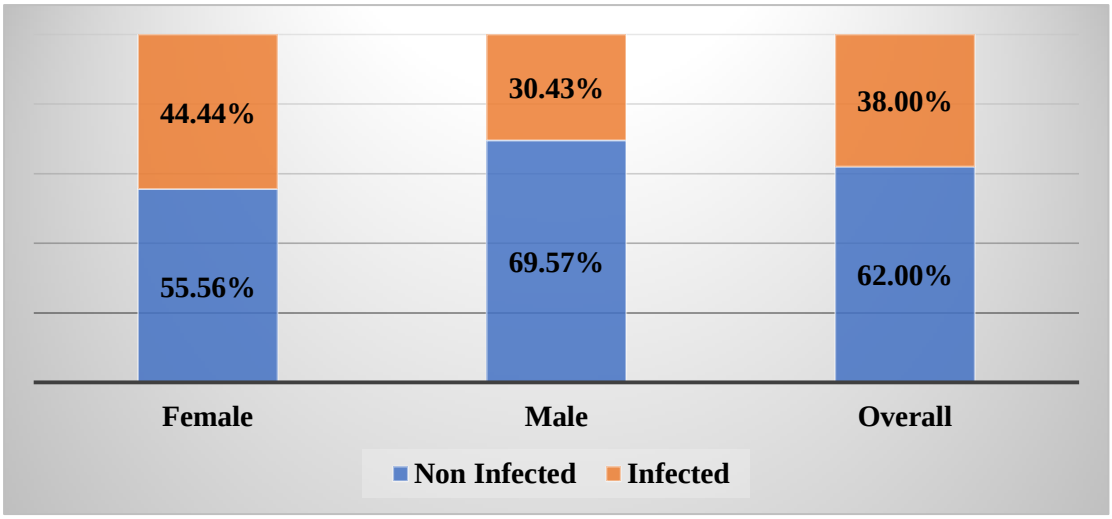

Figure 56. Host sex wise distribution of *N. brasiliensis* infection in *B. bengalensis* collected from railway station

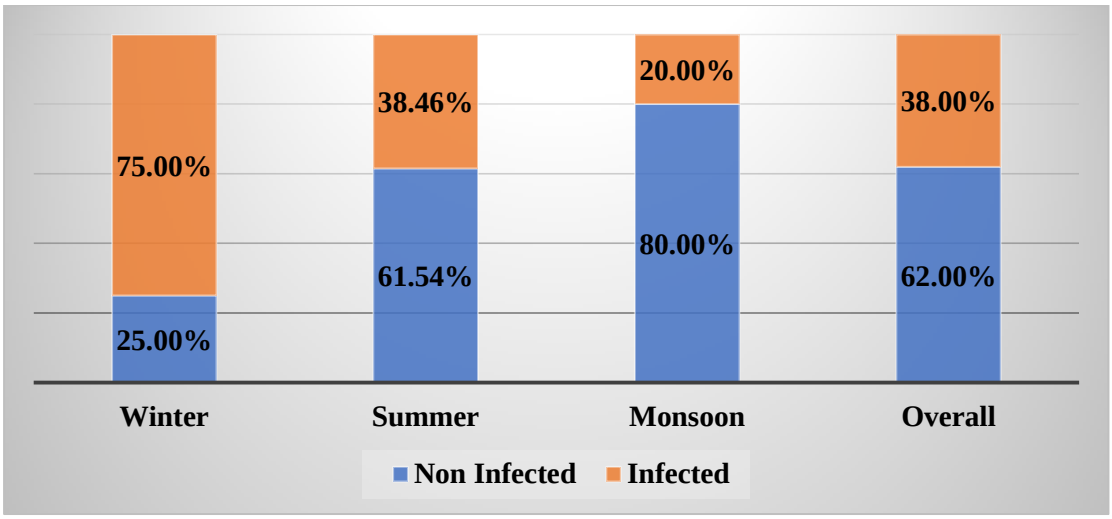

Figure 57. Season wise distribution of *N. brasiliensis* infection in *B. bengalensis* collected from railway station

Table 41: Risk factor analysis of *C. hepaticum* infection in *B. bengalensis* collected from railway station

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Chi square value	P value (d.f.)	95% Confidence interval	
							Lower limit	Upper limit
Age	Young	22	11(50.00)	1.17	0.25	0.61 (1)	0.64	2.12
	Adult	28	12(42.86)	1.00			0.54	1.83
Sex	Female	27	14(51.85)	1.32	0.81	0.37 (1)	0.70	2.47
	Male	23	9(39.13)	1.00			0.48	2.05
Season	Winter	12	7(58.33)	1.82	3.97	0.14 (2)	0.86	3.84
	Summer	13	8(61.54)	1.92			0.94	3.93
	Monsoon	25	8(32.00)	1.00			0.45	2.24
Overall		**50**	**23(46.00)**	-	-	-	-	-

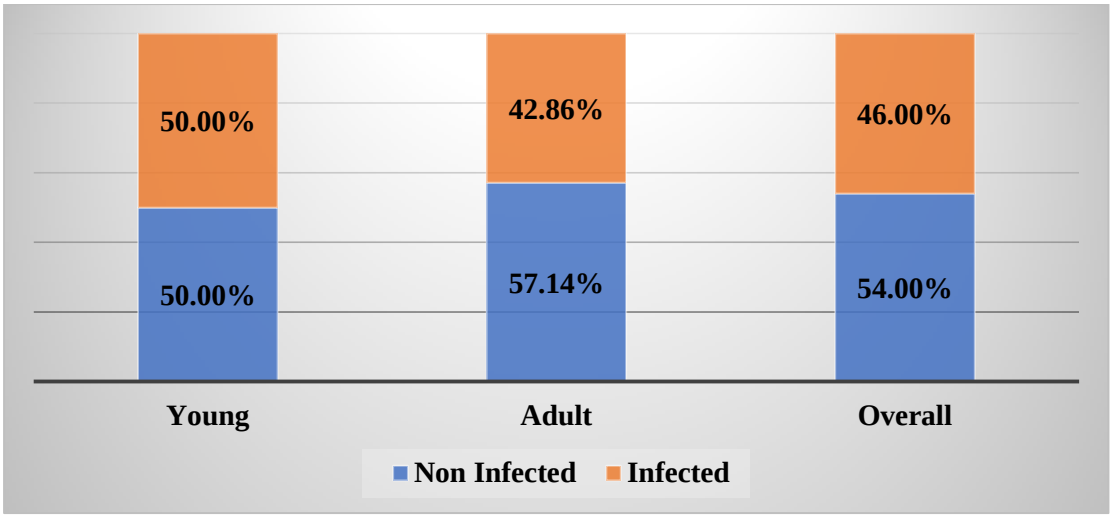

Figure 58. Host age wise distribution of *C. hepaticum* infection in *B. bengalensis* collected from railway station

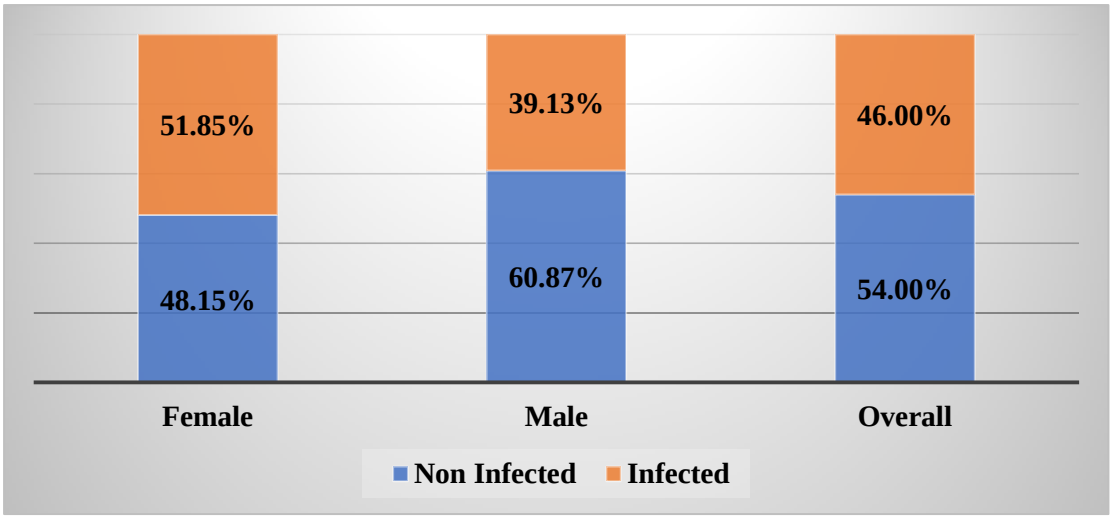

Figure 59. Host sex wise distribution of *C. hepaticum* infection in *B. bengalensis* collected from railway station

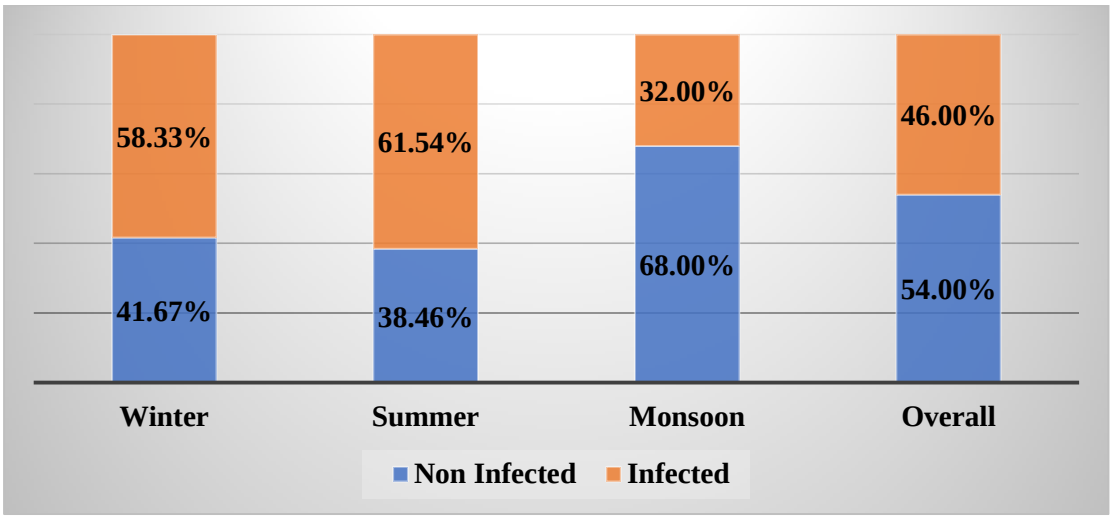

Figure 60. Season wise distribution of *C. hepaticum* infection in *B. bengalensis* collected from railway station

female rats in summer season as compared to adult and male rats in winter and monsoon seasons (Table 41, Figures 58-60).Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of *C. fasciolaris* infection (Table 41).

4.3.2.2.2.3 Analysis of *S. muris*

Out of total 50 rats, only 12.00% (6) were found infected with *S. muris*. Percent infection was higher in summer (16.00%) followed by summer (8.33%) and monsoon (7.69%) seasons. Similarly, percent infection was higher in male (13.04%) and young rats (13.64%) indicating higher relative risk of disease transmission from young and male rats in summer season as compared to adult and female rats in winter and monsoon seasons (Figures 61-63). Total 107 numbers of parasites were recovered from six rats. Prevalence of infection was more in young rats (78.50%) and male rats (71.96%). Prevalence of parasites was high in monsoon season (75.70%) compared to summer (21.49%) and winter (2.80%) seasons. The parasite intensity was high in young and male rats in summer season, while the parasite index was high in young and male rats in monsoon season. Overall mean parasite intensity and parasite index were 17.83 and 2.14, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of *S. muris* infection (Table 42).

4.3.2.2.2.4 Analysis of *T. muris*

Out of total 50 rats, only 10.00% (5) were found infected with *T. muris*. Percent infection was higher in winter (16.67%) followed by monsoon (8.00%) and summer (7.69%) seasons. Similarly, percent infection was higher in female (14.81%) and adult rats (10.71%) indicating higher relative risk of disease transmission from adult and female rats in winter season as compared to young and male rats in monsoon and summer seasons (Figures 64-66). Total 13 numbers of parasites were recovered from five rats. Prevalence of infection was more in adult rats (76.92%) and female rats (69.23%). Prevalence of parasites was high in monsoon season (53.84%) compared to winter (38.46%) and summer (7.69%) seasons. The parasite intensity was high in adult and male rats in monsoon season, while the parasite index was high in adult and female rats in winter season. Overall mean parasite intensity and parasite index were 2.60 and 0.26, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of *T. muris* infection (Table 43).

4.3.2.2.2.5 Analysis of *H. spumosa*

Out of total 50 rats, 30.00% (15) were found infected with *H. spumosa*. Percent infection was higher in winter (33.33%) followed by monsoon (32.00%) and summer (23.08%) seasons. Similarly, percent infection was higher in female (33.33%) and young rats (31.82%) indicating higher relative risk of disease transmission from young and female rats in

Table 42. Risk factor analysis of *S. muris* infection in *B. bengalensis* collected from railway station

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	22	3(13.64)	1.27	84	78.50	28.00	3.81	0.10	0.75 (1)	0.16	1.89
	Adult	28	3(10.71)	1.00	23	21.49	7.66	0.82			0.42	2.34
Sex	Female	27	3(11.11)	1.00	30	28.03	10.00	1.11	0.04	0.83 (1)	0.22	4.52
	Male	23	3(13.04)	1.17	77	71.96	25.66	3.35			0.26	5.26
Season	Winter	12	1(8.33)	1.08	3	2.80	3.00	0.25	0.76	0.68 (2)	0.07	15.46
	Summer	13	1(7.69)	1.00	23	21.49	23.00	1.76			0.06	14.34
	Monsoon	25	4(16.00)	2.08	81	75.70	20.25	3.24			0.25	16.75
Overall		**50**	**6(12.00)**	**-**	**107**	**-**	**17.83**	**2.14**	**-**	**-**	**-**	**-**

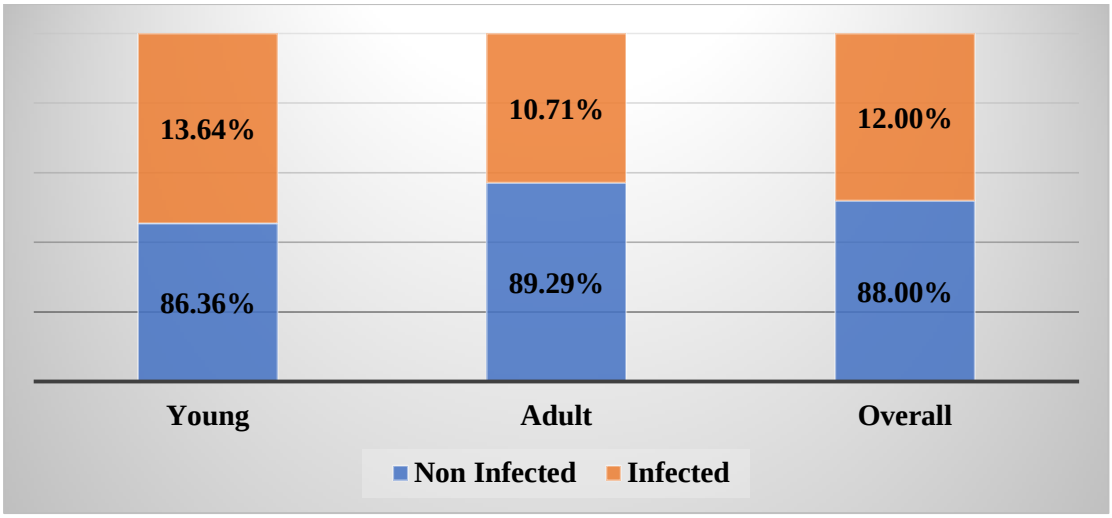

Figure 61. Host age wise distribution of *S. muris* infection in *B. bengalensis* collected from railway station

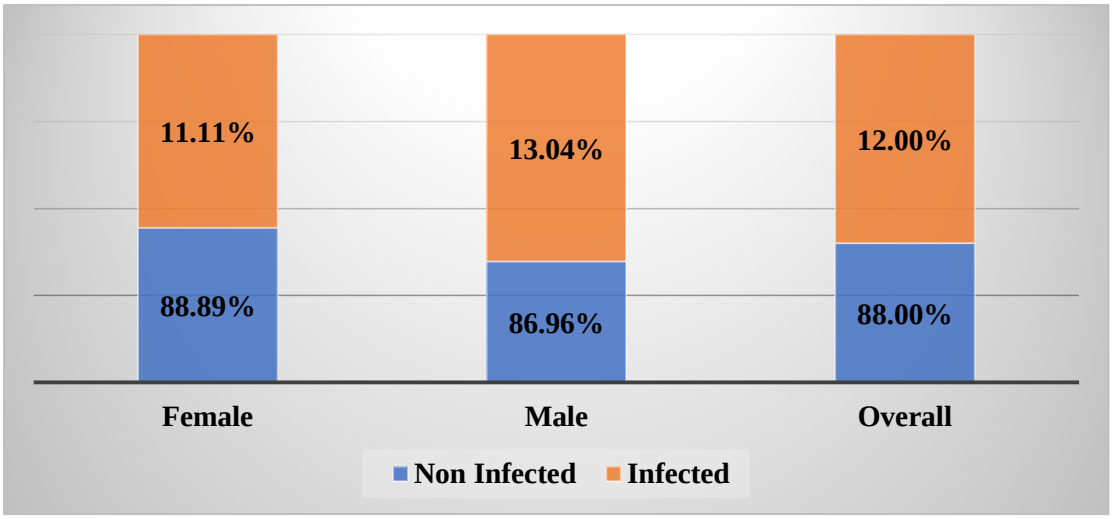

Figure 62. Host sex wise distribution of *S. muris* infection in *B. bengalensis* collected from railway station

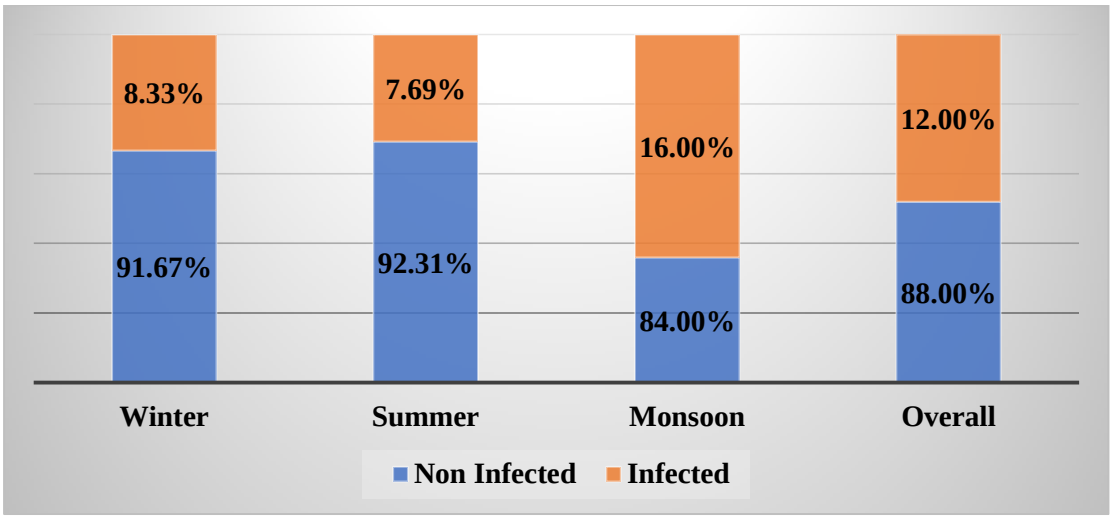

Figure 63. Season wise distribution of *S. muris* infection in *B. bengalensis* collected from railway station

Table 43. Risk factor analysis of *T. muris* infection in *B. bengalensis* collected from railway station

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	22	2(9.09)	1.00	3	23.07	1.50	0.13	0.04	0.85 (1)	0.15	6.48
	Adult	28	3(10.71)	1.18	10	76.92	3.33	0.35			0.19	5.84
Sex	Female	27	4(14.81)	3.41	9	69.23	2.25	0.33	1.51	0.22 (1)	0.40	28.37
	Male	23	1(4.34)	1.00	4	30.76	4.00	0.17			0.06	15.04
Season	Winter	12	2(16.67)	2.17	5	38.46	2.25	0.41	0.78	0.68 (2)	0.22	20.94
	Summer	13	1(7.69)	1.00	1	7.69	1.00	0.07			0.06	14.34
	Monsoon	25	2(8.00)	1.04	7	53.84	3.50	0.28			0.10	10.42
Overall		**50**	**5(10.00)**	-	**13**	-	**2.60**	**0.26**	-	-	-	-

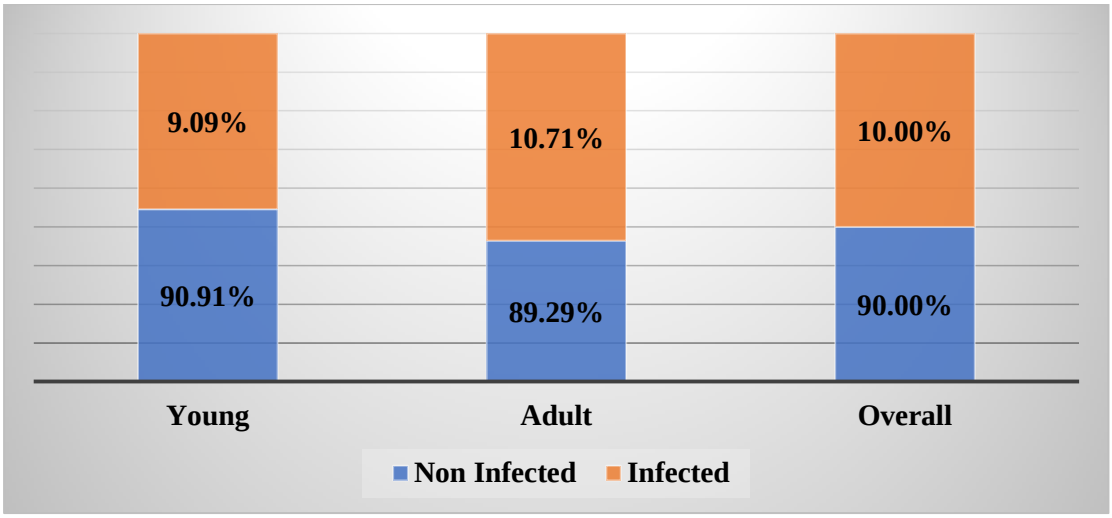

Figure 64. Host age wise distribution of *T. muris* infection in *B. bengalensis* collected from railway station

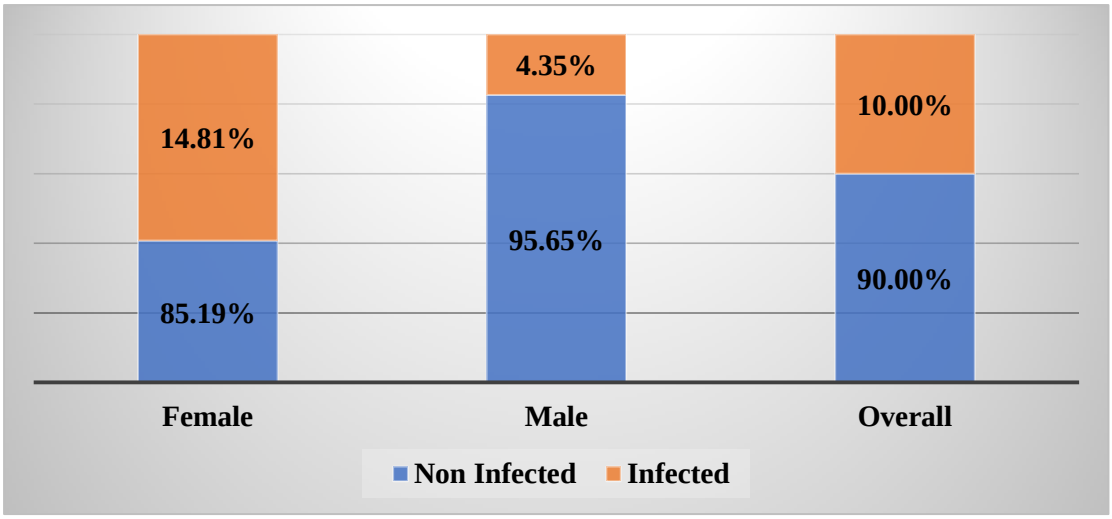

Figure 65. Host sex wise distribution of *T. muris* infection in *B. bengalensis* collected from railway station

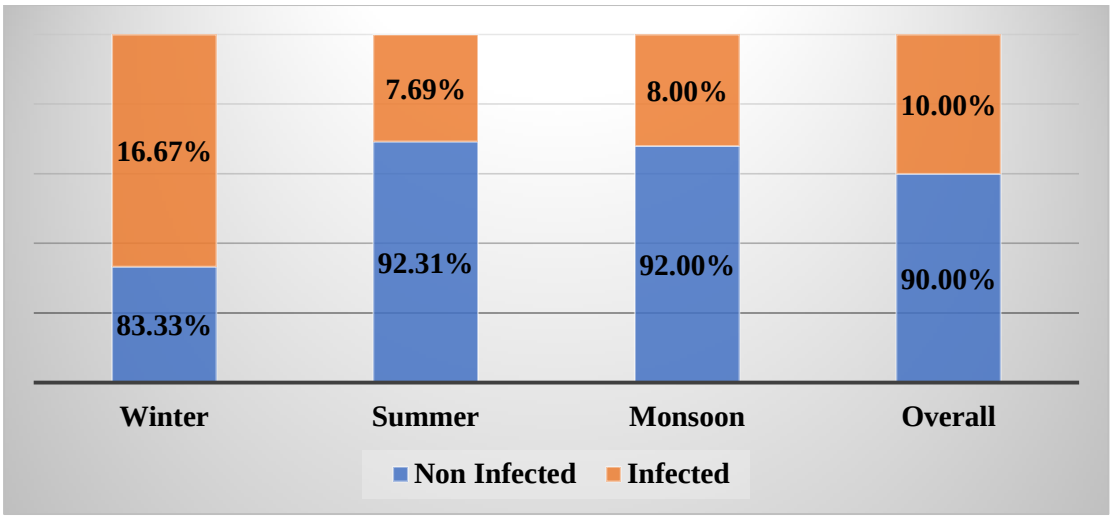

Figure 66. Season wise distribution of *T. muris* infection in *B. bengalensis* collected from railway station

Table 44. Risk factor analysis of *H. spumosa* infection in *B. bengalensis* collected from railway station

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	22	7(31.82)	1.11	43	33.07	6.14	1.95	0.06	0.80 (1)	0.47	2.59
	Adult	28	8(28.57)	1.00	87	66.92	10.87	3.10			0.43	2.28
Sex	Female	27	9(33.33)	1.28	105	80.76	11.66	3.89	0.31	0.58 (1)	0.53	3.05
	Male	23	6(26.09)	1.00	25	19.23	4.16	1.09			0.37	2.64
Season	Winter	12	4(33.33)	1.44	24	18.46	6.00	2.00	0.41	0.81 (2)	0.40	5.16
	Summer	13	3(23.08)	1.00	32	24.61	10.66	2.46			0.24	4.07
	Monsoon	25	8(32.00)	1.39	74	56.92	9.25	2.96			0.44	4.35
Overall		**50**	**15(30.00)**	**-**	**130**	**-**	**8.66**	**2.60**	**-**	**-**	**-**	**-**

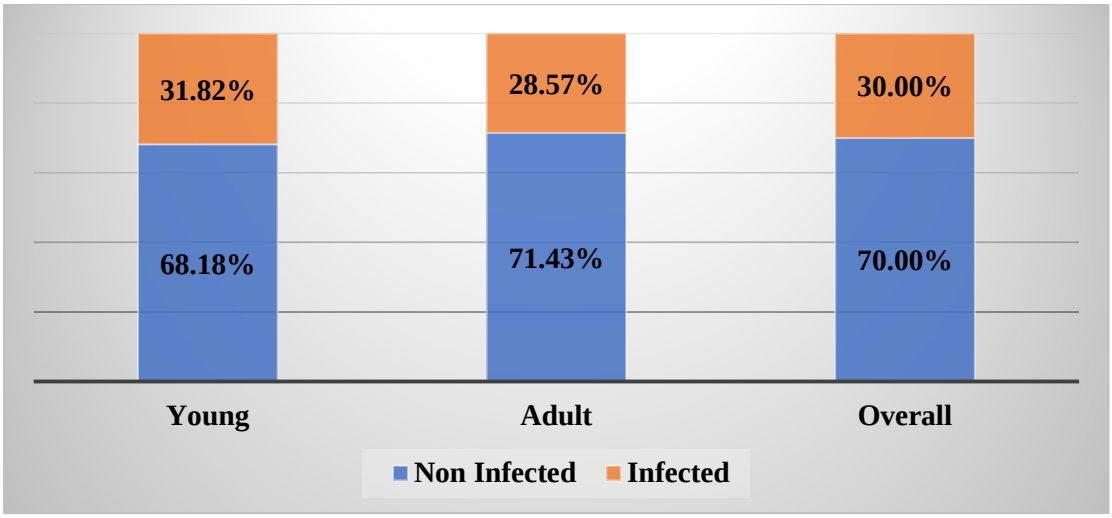

Figure 67. Host age wise distribution of *H. spumosa* infection in *B. bengalensis* collected from railway station

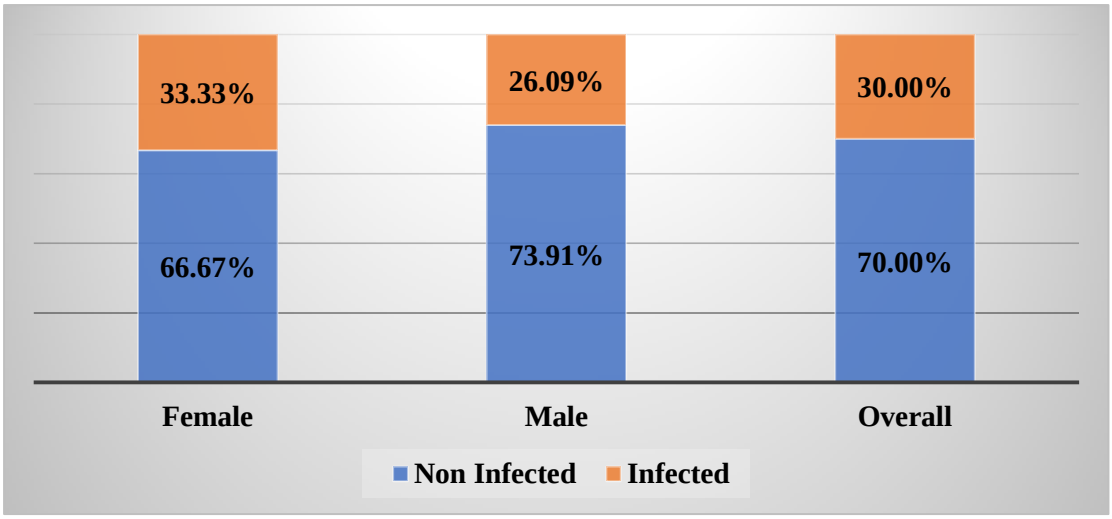

Figure 68. Host sex wise distribution of *H. spumosa* infection in *B. bengalensis* collected from railway station

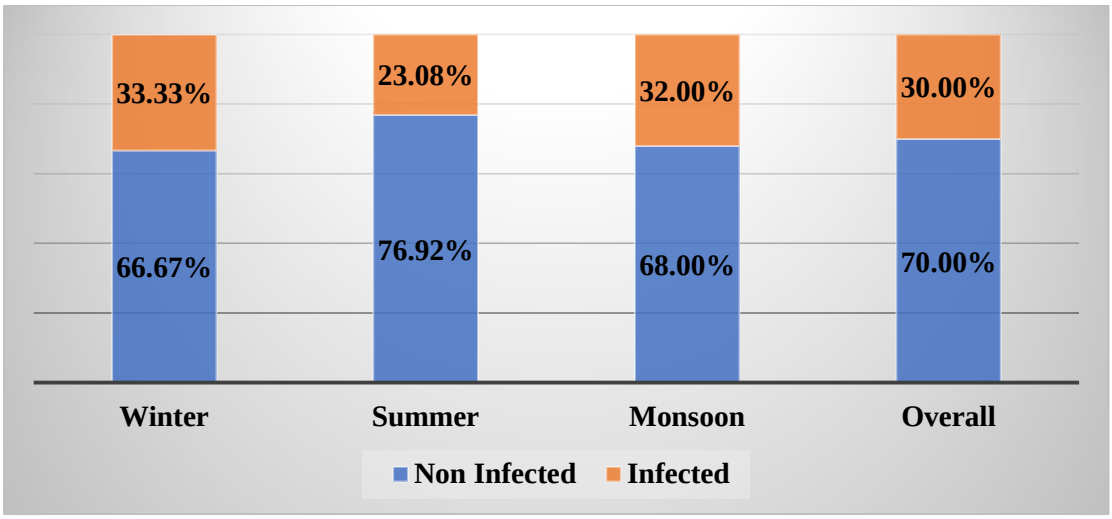

Figure 69. Season wise distribution of *H. spumosa* infection in *B. bengalensis* collected from railway station

winter season as compared to adult and male rats in monsoon and summer seasons (Figures 67-69). Total 130 numbers of parasites were recovered from 15 rats. Prevalence of infection was more in adult rats (66.92%) and female rats (80.76%). Prevalence of parasites was high in monsoon season (56.92%) compared to summer (24.61%) and winter (18.46%) seasons. The parasite intensity was high in adult and female rats in summer season, while the parasite index was high in adult and female rats in monsoon season. Overall mean parasite intensity and parasite index were 8.66 and 2.60, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of *H. spumosa* infection (Table 44).

4.3.2.2.2.6 Overall analysis of nematodes

Overall, out of total 50 rats, 72.00% (36) were found infected with five species of nematodes. Percent infection was higher in winter (91.67%) followed by summer (76.92%) and monsoon (60.00%) seasons. Similarly, percent infection was higher in female (77.78%) and young rats (81.82%) indicating higher relative risk of disease transmission from young and female rats in winter season as compared to adult and male rats in summer and monsoon seasons (Figures 70-72). Total 776 numbers of parasites (except *C. hepaticum*) were recovered from 36 rats. Prevalence of infection was more in young rats (56.96%) and female rats (71.39%). Prevalence of parasites was high in monsoon season (65.21%) compared to summer (21.26%) and winter (13.53%) seasons. The parasite intensity and parasite index were high in young and female rats in summer season. Overall mean parasite intensity and parasite index were 21.55 and 15.52, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of overall nematode infection (Table 45).

4.3.2.2.3 Overall analysis of endoparasites found in rats collected from railway station

Overall, out of total 50 rats, 72.00% (36) were found infected with two species of cestodes and five species of nematodes. Percent infection was higher in winter (91.67%) followed by summer (76.92%) and monsoon (60.00%) seasons. Similarly, percent infection was higher in female (77.78%) and young rats (81.82%) indicating higher relative risk of disease transmission from young and female rats in winter season as compared to adult and male rats in summer and monsoon seasons. Total 836 numbers of parasites (except *C. hepaticum*) were recovered from 36 rats. Prevalence of infection was more in young rats (56.22%) and female rats (70.93%). Prevalence of parasites was high in monsoon season (63.99%) compared to summer (22.01%) and winter (13.99%) seasons. The parasite intensity and parasite index were high in young and female rats in monsoon season. Overall mean parasite intensity and parasite index were 23.22 and 16.72, respectively. Statistical analysis of the data revealed that host age, sex and season had no significant (P>0.05) effect on the prevalence of overall endoparasite infection (Table 46).

Table 45. Risk factor analysis of nematode infection in *B. bengalensis* collected from railway station

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	22	18(81.82)	1.27	442	56.96	24.55	20.09	1.88	0.17 (1)	0.90	1.78
	Adult	28	18(64.28)	1.00	334	43.04	18.55	11.92			0.67	1.47
Sex	Female	27	21(77.78)	1.19	554	71.39	26.38	20.51	0.97	0.32 (1)	0.83	1.71
	Male	23	15(65.21)	1.00	222	28.61	14.80	9.65			0.65	1.52
Season	Winter	12	11(91.67)	1.52	105	13.53	9.54	8.75	4.24	0.12 (2)	1.06	2.19
	Summer	13	10(76.92)	1.28	165	21.26	16.50	12.69			0.82	1.98
	Monsoon	25	15(60.00)	1.00	506	65.21	33.73	20.24			0.63	1.57
Overall		**50**	**36(72.00)**	-	**776**	-	**21.55**	**15.52**	-	-	-	-

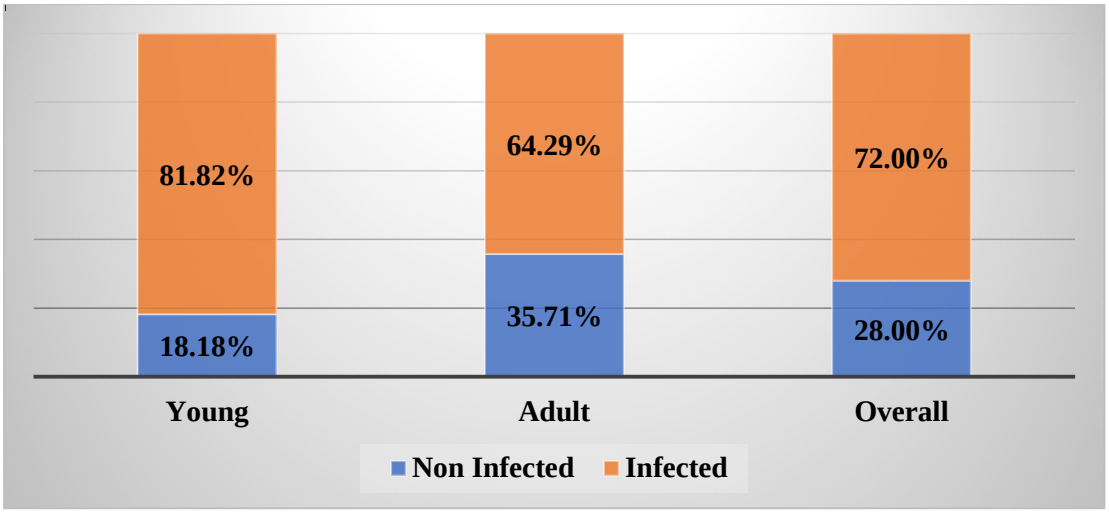

Figure 70. Host age wise distribution of nematode infection in *B. bengalensis* collected from railway station

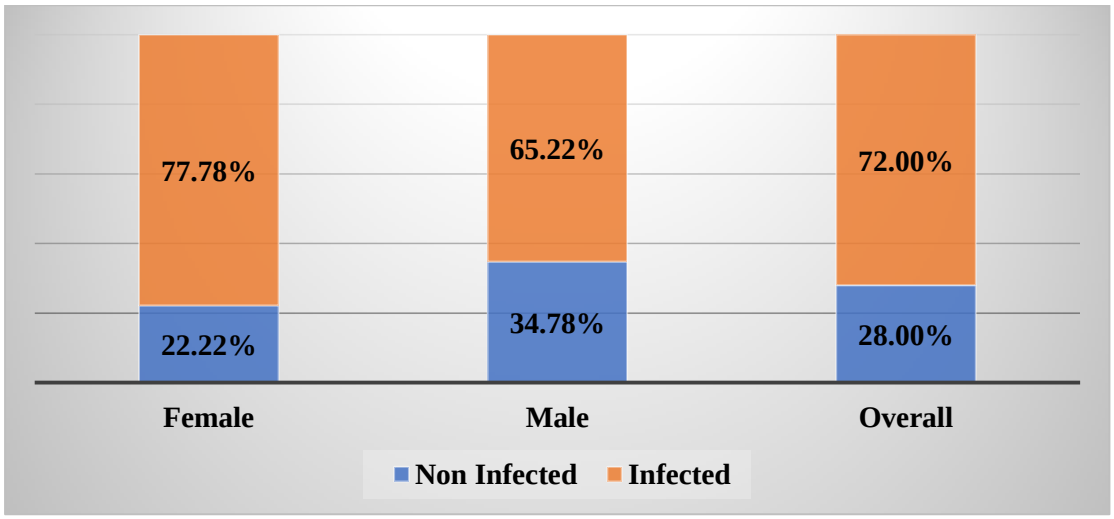

Figure 71. Host sex wise distribution of nematode infection in *B. bengalensis* collected from railway station

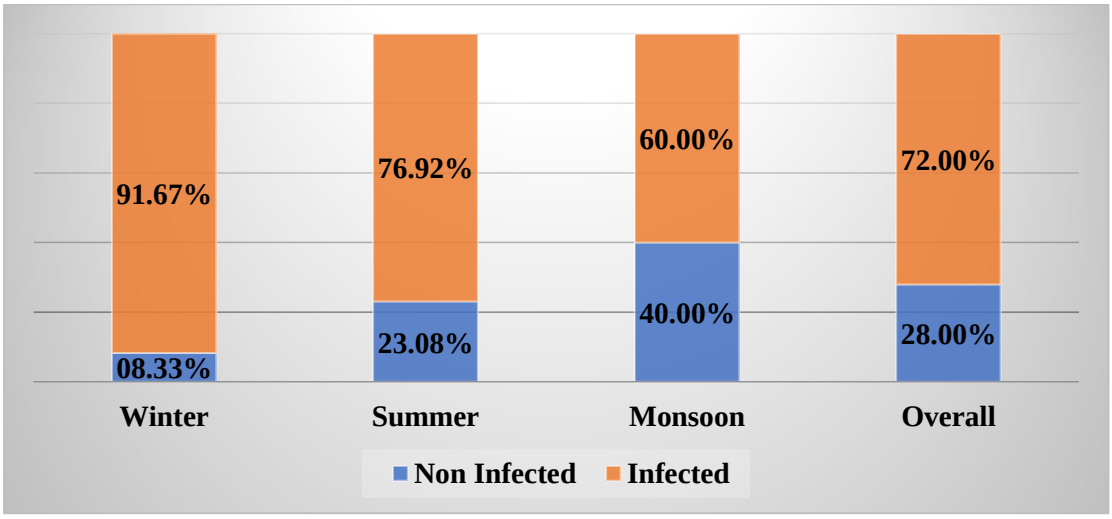

Figure 72. Season wise distribution of nematode infection in *B. bengalensis* collected from railway station

Table 46. Risk factor analysis of overall endoparasitic infection in *B. bengalensis* collected from railway station

Epidemiological factors		Hosts examined	Hosts infected (% infected)	Relative risk	Number of parasites	Parasite prevalence (%)	Parasitic intensity	Parasitic index	Chi square value	P value (d.f.)	95% Confidence interval	
											Lower limit	Upper limit
Age	Young	22	18(81.82)	1.27	470	56.22	26.11	21.36	3.12	0.08 (1)	0.91	1.78
	Adult	28	18(64.28)	1.00	366	43.78	20.33	13.07			0.67	1.47
Sex	Female	27	21(77.78)	1.19	593	70.93	28.24	21.96	1.71	0.19 (1)	0.83	1.70
	Male	23	15(65.22)	1.00	243	29.07	16.20	10.56			0.65	1.52
Season	Winter	12	11(91.67)	1.53	117	13.99	10.64	9.75	5.25	0.07 (2)	1.06	2.19
	Summer	13	10(76.92)	1.28	184	22.01	18.40	14.15			0.82	1.98
	Monsoon	25	15(60.00)	1.00	535	63.99	35.67	21.40			0.63	1.57
Overall		**50**	**36(72.00)**	**-**	**836**	**-**	**23.22**	**16.72**	**-**	**-**	**-**	**-**

4.3.3 Location wise comparison of endoparasitic infection

Total 100 rats (50 each) were collected from two locations. In overall, 79.00% rats were found infected with endoparasites. Total 2428 endoparasites were collected from 79 rats with mean parasite intensity of 30.73 and mean parasite index of 24.28 (Table 47).

In present study, 72.00% of the rats collected from railway station and 86% of the rats collected from fish market at Ludhiana were found infected with different endoparasites. Infection rate of endoparasites in present study was almost similar in male and female rats as well as the young and adult rats. The present results corroborate with the results of Sinniah *et al* (1999) and Kia *et al* (2010) who also reported no significant difference between infection rate in male and female rats. In contrast to the findings of the present study, some workers observed influence of sex on prevalence rate and reported higher prevalence of gastrointestinal helminthes in males than female rats (Paramasvaran *et al* 2005, Onyenwe *et al* 2009, Kataranovski *et al* 2010, Kataranovski *et al* 2011, Solanki *et al* 2013). Similar to present study, Kataranovski *et al* (2011) also did not find effect of host age on any of the nematode Mandla *et al* (2021), however, found a significant effect of host age on parasite infection rate of some nematode parasites.

In present study, though the overall infection rate of endoparasites at both the locations was more in winter season but the parasite prevalence was more in monsoon season. The observations of the present study corroborate with results of Fichet *et al* (2003) reporting higher occurrence of helminths in monsoon season as compared to summer and winter seasons. This may be due to the fact that the climatic conditions such as temperature and rainfall in monsoon season are very much favourable and ideal for the development, dissemination and maintenance of parasitic fauna in the environment (Tuladhar *et al* 2019). In contrast to present study, higher infection of nematode parasites was observed in summer season by Mandla *et al* (2021).

Table 47. Location wise comparison of overall endoparasitic infection in *B. bengalensis*

Location	Host examined	Host infected	Host non-infected	Percent host infected	Number of parasites	Parasite prevalence	Parasite intensity	Parasite index
Fish market	50	43	7	86.00	1592	65.57	37.02	31.84
Railway station	50	36	14	72.00	836	34.43	23.22	16.72
Overall	**100**	**79**	**21**	**79.00**	**2428**	**-**	**30.73**	**24.28**

4.3.4 Morphological features of different endoparasites

4.3.4.1 Cestode parasites

4.3.4.1.1 *H. nana*

The adult tapeworms of *H. nana* were found in small intestine of *B. bengalensis* collected from both the fish market and railway station.Macro and microscopic examination of *H. nana* revealed tapeworms to bedorso-ventrally flattened, 2-4 cm long having scolex with four suckers and a retractable rostellum armed with a crown of 20-30 hooks. The strobila was typical with about 200 segments, reproductive organs and gravid proglottids containing a large number of eggs (Plate 2). Numerous eggs of *H. nana* were found in faecal samples. The eggs were oval and small. The oncosphere of the egg had six hooks. Hexacanth embryo within the oncosphere contained 4-8 polar filaments (Plate 2). Eggs measured 58.10-62.25 μm in length and 41.50-49.80 μm in breadth (Table 48). Similar kind of eggs of *H. nana* was also reported by Whary *et al* (2015) and Brar *et al* (2021a). The eggs of both of these species 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.

4.3.4.1.2 *C. fasciolaris*

The *C. fasciolaris,* a metacestode (larva) of *T. taeniaeformis* was found in the form of whitish cysts on the liver. Mostly one cyst was found in each animal during the present study except one rat in which more than one cyst were found. Each cyst contained single live characteristic strobilocercus larva (Plate 3). The metacestode had an armed scolex with two rows of hooks and lateral suckers. Concurrent infection of *C. fasciolaris* and C. *hepaticum* was observed in most of the rats. The size of *C. fasciolaris* cysts varied from 4-12 mm in diameter. The larvae measured 10-18 cm in length. The occurrence of *C. fasciolaris* has been reported in laboratory and wild rodent species by many workers in India (Jithendran and Somvanshi 1999, Singla *et al* 2003, Singla *et al* 2013, 2016, Brar *et al* 2021). Similar to present results, concurrent infection of *C. fasciolaris* and C. *hepaticum*in liver was also reported earlier by Gotardo *et al* (2000), Raut *et al* (2003), Mowat *et al* (2009), Li *et al* (2010), Singla *et al* (2013) and Zamini *et al* (2017).

4.3.4.2 Nematode parasites

4.3.4.2.1 *N. brasiliensis*

This nematode was found to inhabit small intestine of *B. bengalensis*. Both male and female parasites were recovered. It showed sexual dimorphism as male had characteristic bursa at the posterior end which forms an umbrella like expansion around the cloaca (Plate 4). Females were longer than males. The vulva was present towards posterior end that opened in front of the anus. Ovary was filled with a single row of developing oocytes (Plate 4). The uterus occupied much of the posterior part of the body. The eggs were thin shelled and ellipsoidal and measured 62.25-74.70 μm in length and 33.20-37.35 μm in breadth (Table 48).

4.3.4.2.2 *C. hepaticum*

The *C. hepaticum* infestation was found in the liver. Gross lesions comprising of irregular pale cystic areas were found randomly scattered on the liver surface of rodents (Plate 5). No adult worms were found in the liver. On further investigation, it became clear that these rats were infected with *C. hepaticum*. Large deposition of eggs was found in the liver tissue of rats infected with *C. hepaticum*. Typical eggs with bipolar caps were found scattered in the parenchyma of the liver (Plate 5). The eggs measured 62.25-66.40 µm in length and 33.20-37.35 µm in breadth. Wright (1961) measured *C. hepaticum* eggs to be of size 54×31 µm on average, whereas, Li *et al* (2010) measured them to be about 48-66×28-36 µm (Table 48).

4.3.4.2.3 *S. muris*

This nematode was found infecting the large intestine of *B. bengalensis*. Females were longer than males. They had characteristic oesophageal bulb at the anterior region. The adult worm was cylindrical, with rounded anterior end and tapered pin like posterior end (Plate 6). The mouth was surrounded by three distinct lips. The male worm had a single long prominent spicule at the posterior region. The female worm had vulva in the anterior quarter of the body. The present study was in accordance with Khatoon *et al (2004),* Meade and Watson (2014), BinoSundar *et al* (2018), in which *S. muris* were recovered from *R. norvegicus*. During the present study, a number of eggs of *S. muris* were found in the faecal samples of *B. bengalensis* collected from two locations. The eggs were asymmetrical, vermiform, slightly flattened on one side and pointed at both the sides (Plate 6). Eggs measured 83.00-87.15µm in length and 24.90-29.05 µm in breadth (Table 48). Similar to present study, Sharma *et al* (2013) and Meade and Watson (2014) also found the eggs of *S. muris* in the faecal samples of rats collected from domestic places.

4.3.4.2.4 *T. muris*

It was a small nematode of 1-2 cm length with cylindrical non-segmented body. Adult *T. muris* males had coiled posterior end and adult females had straight posterior end (Plate 7). Both sexes had a long, whip-like anterior end. The anterior end contained the mouth and oesophagus while the posterior end contained the anus and sex organs. The present study is comparable to the studies conducted by Smith and Carpenter (2006), Elsheikha *et al* (2008), Callejon *et al* (2010) and Wasimuddin *et al* (2016). A number of eggs of *T. muris* were recovered from the faeces of *B. bengalensis*. Under microscope, the eggs were found to be thick shelled with bipolar plugs at both ends (Plate 7). Eggs measured 66.40-74.70 µm in length and 33.20-37.35 µm in breadth (Table 48).

4.3.4.2.5 *H. spumosa*

The worms of *H. spumosa* were recovered from large intestine of rats. Both male and female adult parasites of about 1cm length were found (Plate 8). At the anterior end, mouth with distinct lips and at the posterior end, anus was found. Adults showed marked sexual dimorphism. Males were shorter with copulatory bursa at the posterior end, while females

were longer with pointed posterior end. Thick-walled eggs of *H. spumosa* were found in the faecal samples (Plate 8). Eggs measure 53.95-70.55 µm in length and 29.05-41.50 µm in breadth (Table 48).

Table 48. Dimensions of eggs of different endoparasites found in faecal samples of rodents

Endoparasite species	Length (µm)		Breadth (µm)	
	Range	Mean±SD	Range	Mean±SD
H. nana	58.10-62.25	59.48±1.96	41.50-49.80	45.65±3.39
N.brasiliensis	62.25-74.70	69.17±5.17	33.20-37.35	35.97±1.96
C.hepaticum	62.25-66.40	65.02±1.96	33.20-37.35	35.97±1.96
S.muris	83.0-87.15	84.38±1.96	24.90-29.05	27.67±1.96
T.muris	66.40-74.70	70.55±3.39	33.20-37.35	34.58±1.96
H.spumosa	53.95-70.55	63.63±7.05	29.05-41.50	34.58±5.18

4.3.5 Molecular identification on *N. brasiliensis*

On the basis of molecular characterization, the nematode parasite species was identified as *N. brasiliensis.* The mitochondrial cytochrome oxidase I (COI) gene of *N. brasiliensis* was successfully amplified. The PCR amplification of this region showed a single band of approximate size of 355 bp (Figure 73). The Blast results showed that the sequence of the nematode is closer to those of species of genus *Nippostrongylus*, with 100% similarity with sequence of *N. brasiliensis* available in GenBank (AP017690) from Japan.

Multiple alignment of COI region of query sequence (*N. brasiliensis* rom Punjab, India) with three different geographical isolate species i.e., *N. brasiliensis* from Japan(Accession number AP017690), USA (Accession number U57035) and New Zealand (Accession number NC_033886) showed 100.00% similarity (Figure 74). When compared with other sequences of related species across the globe, COI gene of Indian isolate revealed variable nucleotide homologies with a range of 88.18-100.00%. The Nucleotide sequence data of COI of *N. brasiliensis* reported in this paper has been submitted to the GenBank with the accession number LC627506.

The phylogenetic tree based on COI gene region revealed all the taxa into two major clades (Figure 75). The Genus *Nippostrongylus* formed a large clade in which *N. brasiliensis* (Punjab, India) remained in the same cluster together with *N. brasiliensis* from USA, New Zealand and Japan and supported by high bootstrap value. Other hook worms such as *Necator americanus* (Brazil), *Heligmosomoides polygyrous* (USA) and *H. polygyrous* (Turkey) constitute the second major clade. This analysis involved nine nucleotide sequences. The tree

was drawn to scale with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated using complete deletion option. There was a total of 310 positions in the final dataset. Similar molecular identification of *N. brasiliensis* by PCR amplification of mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene was also been done by Chaudhary *et al* (2016).

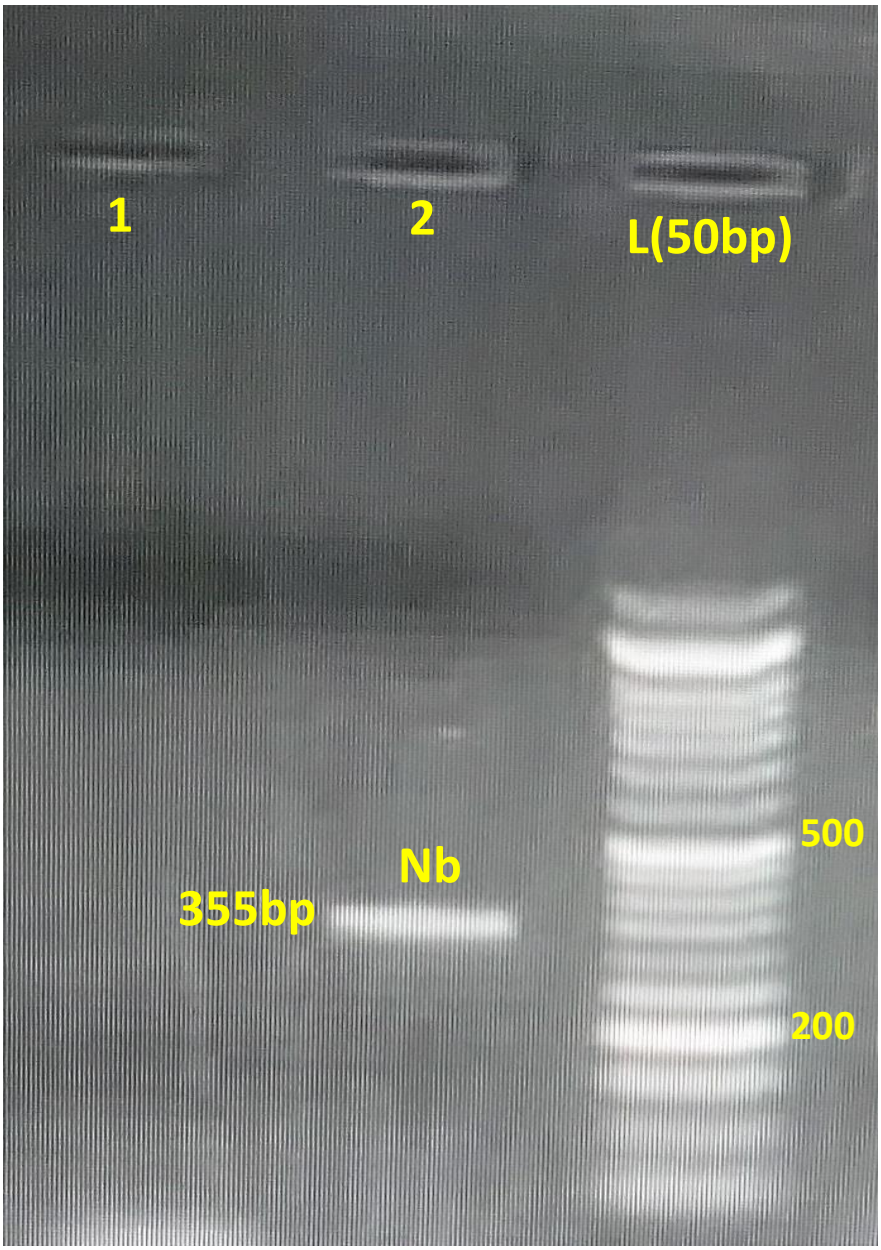

Figure 73. The PCR amplification of COI region of *N. brasiliensis*. Lane M: 100 bp DNA ladder. Lane 1: Positive sample with band size of 355bp, and Lane 2: Negative control

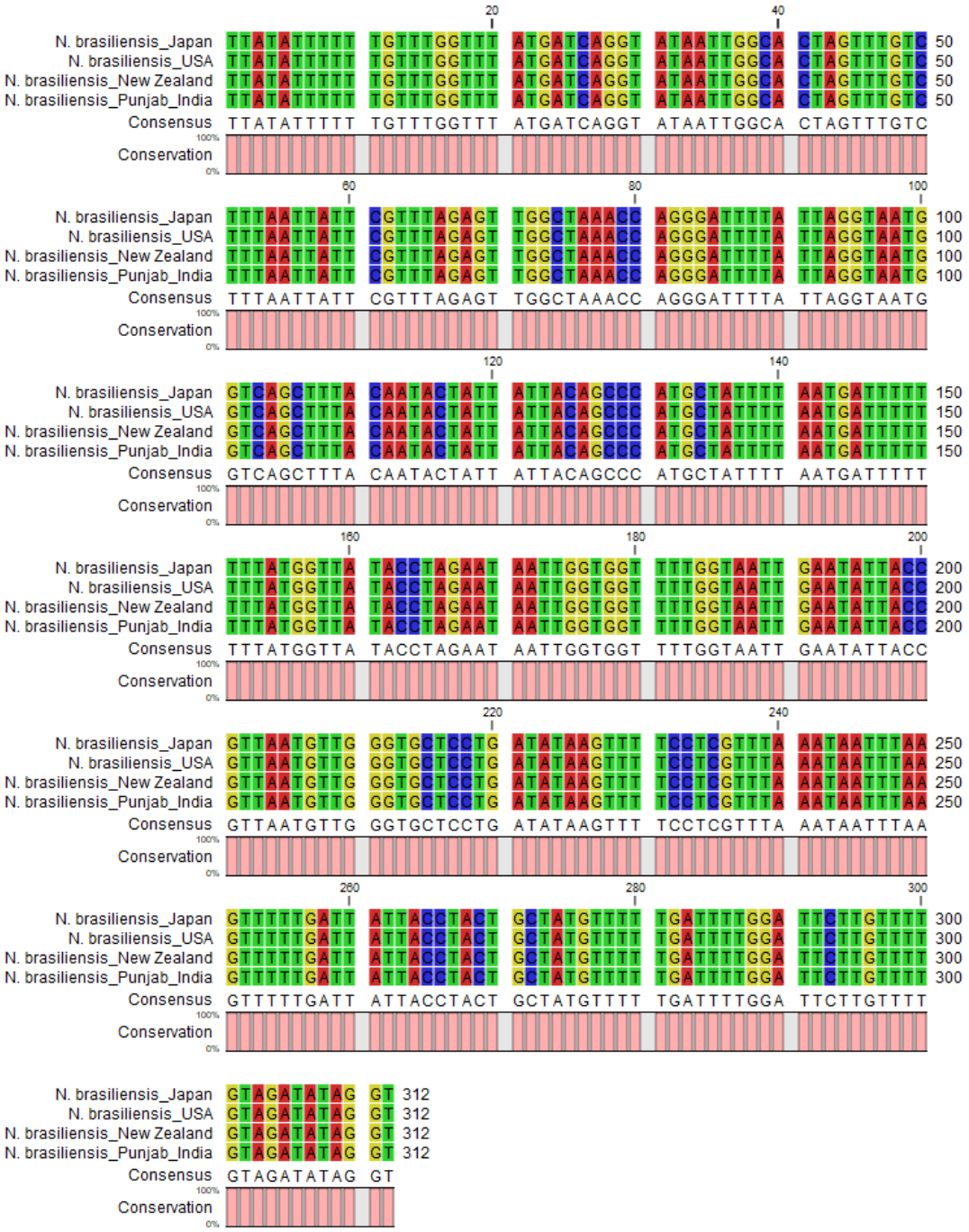

Figure 74. Multiple alignment of the query *N. brasiliensis* sequence with different geographical isolates

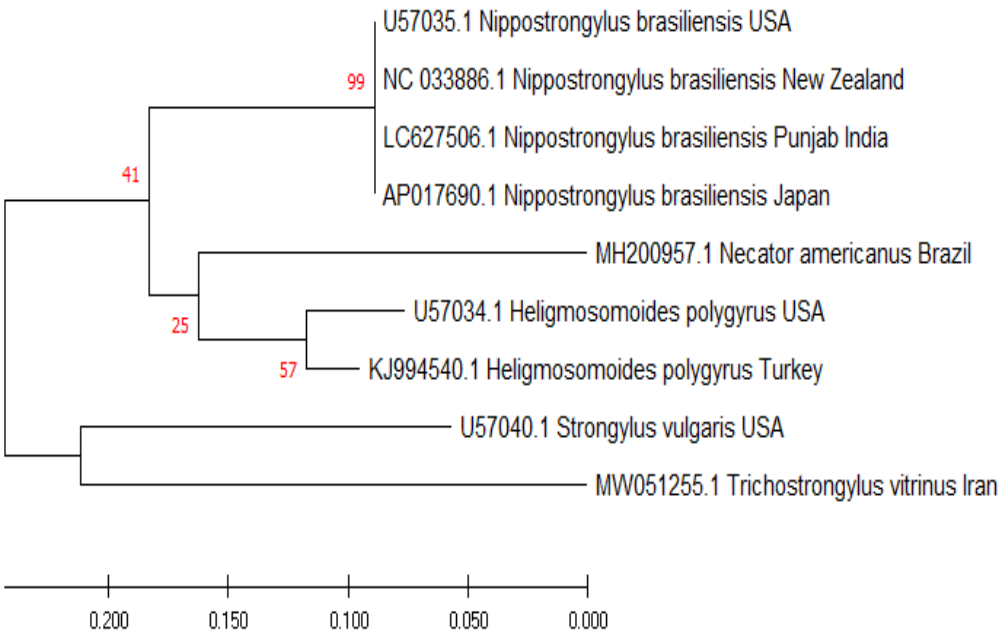

Figure 75: Phylogenetic tree of *N. brasiliensis* constructed using maximum likelihood method

4.3.6 Zoonotic potential of different endoparasites

In present study 24-34% *B. bengalensis* were found infected with *H. nana*. Similar rates of infection of *H. nana* (28.80-38.00%) have also been reported in other studies (Kassan and Assefa 2000, Stojcevic *et al* 2004, Abu-Madi *et al* 2005, Kumarasinghe *et al* 2006, Paramasvaran *et al* 2009, Ranjbar *et al* 2017). Infection rates higher (50.66-56.70%) than those found in present study have also been reported in some studies (Gilioli *et al* 2000, Rasti*et al* 2000, Tanideh *et al* 2010). However, in contrast to present study, much lower (11.00-12.50%) prevalence rates of *H. nana* have been reported by many researchers in other countries (Webster and MacDonald 1995, Kia *et al* 2001).

Brar *et al* (2021a) found concurrent infection of *H. nana* and *H. diminuta* in *B. bengalensis*, but in present study rats were found infected with only *H. nana*. Hymenolepiasis due to *H. nana* and *H. diminuta* has been reported to affect about 36 million people worldwide (Peters and Pasvol 2002, Watwe and Dardi 2008, Patamia *et al* 2010, Panti-May *et al* 2020). The disease is more common in areas where structural and socio-environmental conditions are poor and where there is close contact between rodents and humans. Both species of cestodes are cosmopolitan parasites that have a wide range of prevalence values. These species have been recorded in *B. bengalensis* in different environments, but mostly in urban areas (Battersby *et al* 2002, Mohd Zain *et al* 2012, Ahmad *et al* 2014, Singla *et al* 2016). *Hymenolepis* spp. are considered to be of public health importance because of their detrimental pathologies associated with human infections. Humans get easily infected by

these parasites, as rat's co-habitat their homes (Onyenwe *et al* 2009, Alvarez-Fernández *et al* 2012, Karuna and Khadanga 2013, Kandi *et al* 2019, Goudarzi *et al* 2021). It is estimated that more than 21 million people around the world suffer from these infections and most of them are from the tropical and subtropical regions (Parija 1990). 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 (Abu-Madi *et al* 2005). Humans can accidentally enter into the life cycle of this tapeworm via the ingestion of infected insects (intermediate hosts) containing cestode cysticercoids in their body cavity (Panti-May *et al* 2020). Young children can be infected with *H. nana* eggs from rodent sources (Hosseini *et al* 2015) and are at greater risk.

*T. taeniaeformis*is an intestinal tapeworm reported in both domesticated and wild cats and related carnivores whereas, its larval form, *C. fasciolaris* is predominant in the rodents that serve as its intermediate hosts (Iwaki *et al* 1994). Rodents are infected by ingesting the ova in contaminated soil and bedding material (Jithendran and Somvanshi 1998). The larvae developed inside the body migrate to liver, become strobilocercus or metacestode and get encysted as a chronic infection (Jithendran and Somvanshi 1998, Singla *et al* 2003). *C. fasciolaris* infection is clinically asymptomatic and is considered harmless. The adult worm is frequently encountered in the small intestine of the cat and wild felids and infrequently in the dog and other carnivores (Singla *et al* 2009). In present study 12-20% rats were found infected with *C. fasciolaris* in the form of cysts on the liver. Human infection with this parasite was first reported in a 5-year-old boy from Buenos Aires (Bacigalupo 1922). In humans, there are a number of records of the occurrence of adult (Ekanayake *et al* 1999, Moudgil *et al* 2013) and larval (Sterba *et al* 1977) forms of *T. taeniaeformis.* However, this cestode is considered to pose a low health risk.

The nematodes recorded during the present study are also reported to have zoonotic importance (Waugh *et al* 2006, Singla *et al* 2008a, Paramasvaran *et al* 2009, Kia *et al* 2010). *Calodium* spp. are the most important nematodes of rodents as far as zoonosis is concerned (Onyenwe *et al* 2009, Paramasvaran *et al* 2009, Kia *et al* 2010). Although rare, *C. hepaticum* is responsible for hepatic capillariasis in humans from different parts of the world (Fuehrer 2014). In India, the human infection by *C. hepaticum* was sporadically reported from a soldier (Sinniah*et al* 1979). Although rare, *C. hepaticum* is responsible for Hepatic capillariasis, a disease most common in rodents but is rare in humans. Up to now, 72 cases of *C. hepaticum* infection in humans have been reported worldwide since the first case was described by MacArthur in 1924 (MacArthur 1924, Fuehrer *et al* 2011). In an urban area of Brazil, 1.8% residents were found infected with *C. hepaticum* (Rocha *et al* 2015). There is a case report of severe infection of *C. hepaticum* in a 5-year-old child of Iran (Aghdam *et al* 2015). In wild rodents, the eggs of *C. hepaticum* may be liberated from the liver into the external

environment (soil/water) by the natural death of the host and decomposition of the body, from where it can infect humans and other animals (Spratt and Singleton 2001). After ingestion by definite host, the embryonated eggs hatch in the small intestine or caecum, penetrate the intestinal mucosa and pass to the liver via the portal or mesenteric veins within 2-3 days post infection. The final moulting of the larva to produce the male or female adults occurs at 18-20 days post-infection (Spratt and Singleton 2001). The female worms after fertilization, deposit eggs in the liver and subsequently, the adult worms start dying and disintegrating.

In present study, 38-48% *B. bengalensis* were infected with *N. brasiliensis* with very large number (1439) of adult parasites recovered from small and large intestine. The parasite intensity and parasite index ranged from 27.68-38.04 and 10.52-18.26, respectively indicating high risk of disease transmission. Coomansingh *et al* (2009) and Mandla *et al* (2021) also reported that *N. brasiliensis* infection in rodents was much more than any other endoparasite infection which is in agreement with our present study. With *N. brasiliensis* nfection, there is T-cell mediated immune response stimulation in the host resulting in expulsion of worms, but this phenomenon fails to develop when infection occurs in rats (Wakelin 1996). This could be the reason why *N. brasiliensis* infection was more in rats.

The nematode, *S. muris* is a short lived (7-8 days) parasite having direct life cycle. The eggs are deposited by the female in the colon, where they embryonate within hours. Apart from the usual oral mode of infection, autoinfection is also common. Parasitic infestation is generally considered to be non-pathogenic (Taffs 1976). In present study infection rate of *S. muris* was found to be 12-14%. Similar to our results, Jarosova *et al* (2020) also reported 19.3% infection rate of *S. muris* in rats.

Trichuris spp., the etiological agent of trichuriasis, has parasitized many domesticated species causing enteritis, diarrhoea and weight loss (Souls by 1982). Adult worms of *T. muris* live in the caecum where they produce thousands of eggs with characteristic bi-polar plugs. Eggs are passed in the host's faeces and become infective within 2-3 weeks. Animals get infected by the ingestion of infective eggs (Zajac and Conboy 2012) which hatch in the small intestine and the larvae migrate to the large intestine where it reaches sexual maturity (Aiello and Mays 1998, Smith and Carpenter 2006). Over 500 million cases of human trichurioses have been reported worldwide (Pullan *et al* 2014) and these cases have been most probably attributed to infections with *T. muris.*

H. spumosa is usually a parasite of rats, mice and occasionally of hedgehogs (Klimpel *et al* 2007, Ribas *et al* 2013, Snabel *et al* 2014). Infection by these nematodes occurs by oral uptake of embryonated eggs that hatch in the stomach. The larvae then migrate to the caecum and colon where they develop and become mature. Unembryonated eggs are passed in the faeces 26 to 47 days after infection and become infectious in 14 days under optimal conditions (Smith 1953). Globally, the prevalence of *H. spumosa* used to be high particularly

in *R. norvegicus* (Milazzo *et al* 2010). It was frequently encountered in rats in the Pampean region of Argentina (66%) (Gomez Villafane *et al* 2008), in Palermo at Sicily (82.5 %) (Milazzo *et al* 2010) and in the Samsun district of northern Turkey (79.4 %) (Gurler*et al* 2011). In Poland, Zalesny *et al* (2010) reported 72.7% prevalence of *H. spumosa* in *Apodemusagrarius* in two localities in Lower Silesia.

The co-existence of *B. bengalensis* with diverse parasitic species observed in present study indicates host's capability to support parasites' nutritive and developmental needs. Despite heavy infection with intestinal parasites (such as *N. brasiliensis, S. muris* and *H. spumosa*) and marked hepatic tissue damage due to severe infection of *C. hepaticum* and *C. fasciolaris* infection, all rats appeared healthy and active suggesting a well-established host-parasite relationship. In view of the diversity and zoonotic nature of rat parasites and the conditions prevailing in commensal habitats where *B. bengalensis* survive and proliferate, they can readily facilitate parasite transmission to humans and other susceptible animals. Similar role of *Rattu*s spp. in transmitting zoonotic diseases and strong host-parasite relationship has also been suggested by Claveria *et al* (2005).

The present study thus concludes that:

- In overall, 25.00% rats collected from both the locations were found infected with rat fleas with mean flea intensity of 1.36 and mean flea index of 0.34. The overall flea index of <1.0 indicates that there is low risk of disease transmission from rat fleas.
- Rats collected from both the locations were found infected with total seven species of endoparasites, comprising two species of cestodes i.e., *H. nana* and *C. fasciolaris* and five species of nematodes i.e., *N. brasiliensis, C. hepaticum, S. muris, T. muris* and *H. spumosa*.
- The endoparasites were found at multiple locations in the host like in liver, small intestine and large intestine. Rats were found to have concurrent infection of two or more species of parasites. But all the animals were active.
- Identity of *N. brasliensis* was also confirmed by molecular characterization.
- Prevalence of infection in rats collected from fish market was more in young (66.33%) and female rats (62.00%) in winter season (55.21%). Prevalence of infection in rats collected from railway station was more in young (56.22%) and female rats (70.93%) in monsoon season (63.99%).
- Out of total 100 rats collected from two locations, 79.00% were found infected with endoparasites. Total 2428 number of endoparasites were collected from 79 rats with mean parasite intensity of 30.73 and mean parasite index of 24.28 indicating high risk of disease transmission.

The study thus indicates *B. bengalensis* inhabiting commensal areas to be risk associated as being loaded with number of ecto- and endoparasites. The close association of these rats to human dwellings may facilitate easy transmission of these zoonotic parasites. Being nocturnal, the rats are very active during night hours, wandering here and there and contaminating the environment, food and water thus responsible for spread of diseases of veterinary and zoonotic importance. Present study suggests that proper rodent pest and vector management should be conducted in animal and human inhabitations to avoid the spread of zoonotic diseases caused by them.

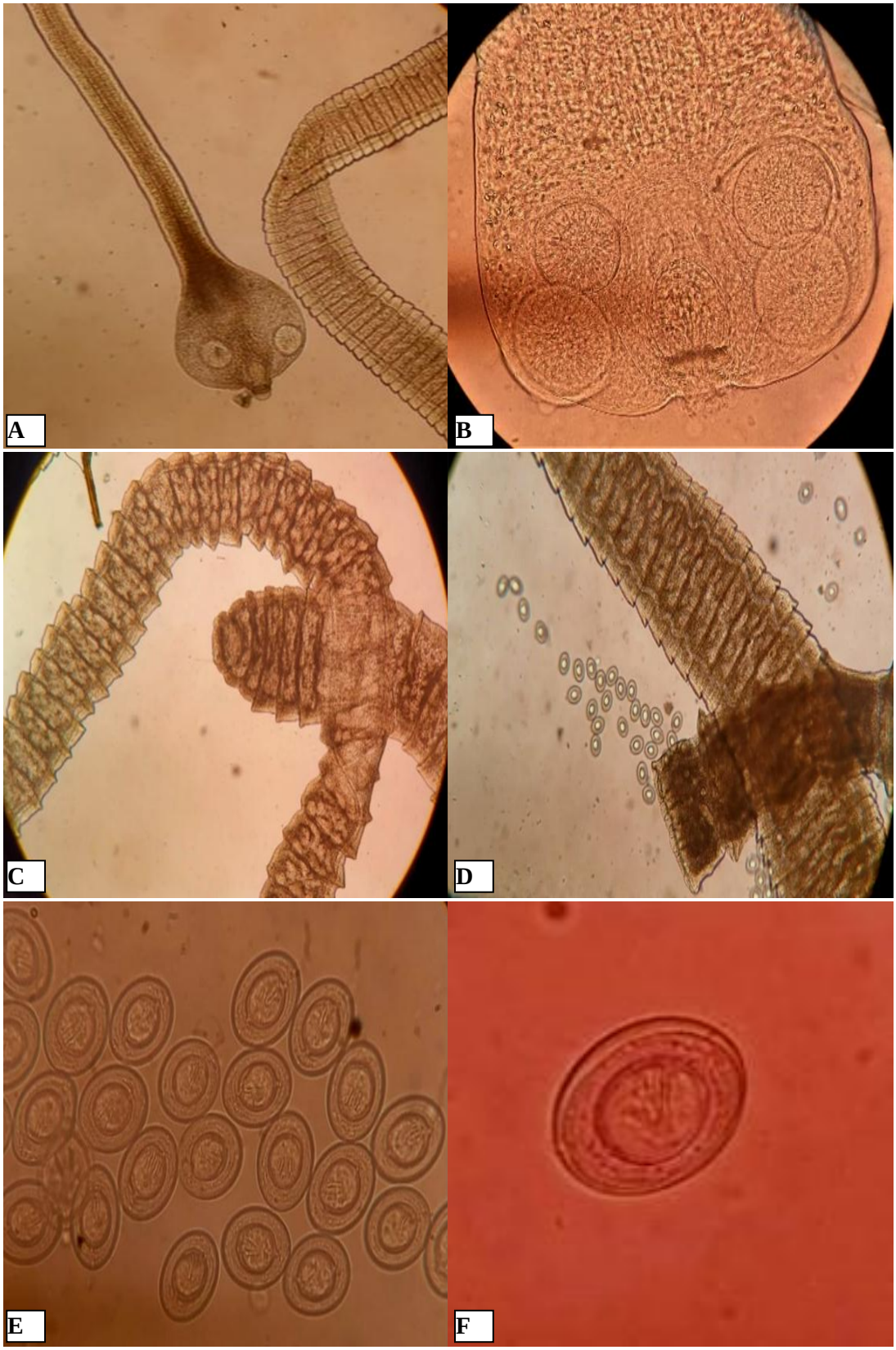

Plate 2. ***Hymenolepis nana*** **found in small intestine of** ***Bandicota bengalensis,*** **A. Worm with scolex, neck and strobila, B. Scolex having four suckers and retractable rostellum, C. Strobila having proglottids, D. Eggs released from mature proglottids, E. Multiple eggs released from mature proglotids, and F. Egg found in faecal sample and showing hexacanth embryo in onchosphere at 400x magnification**

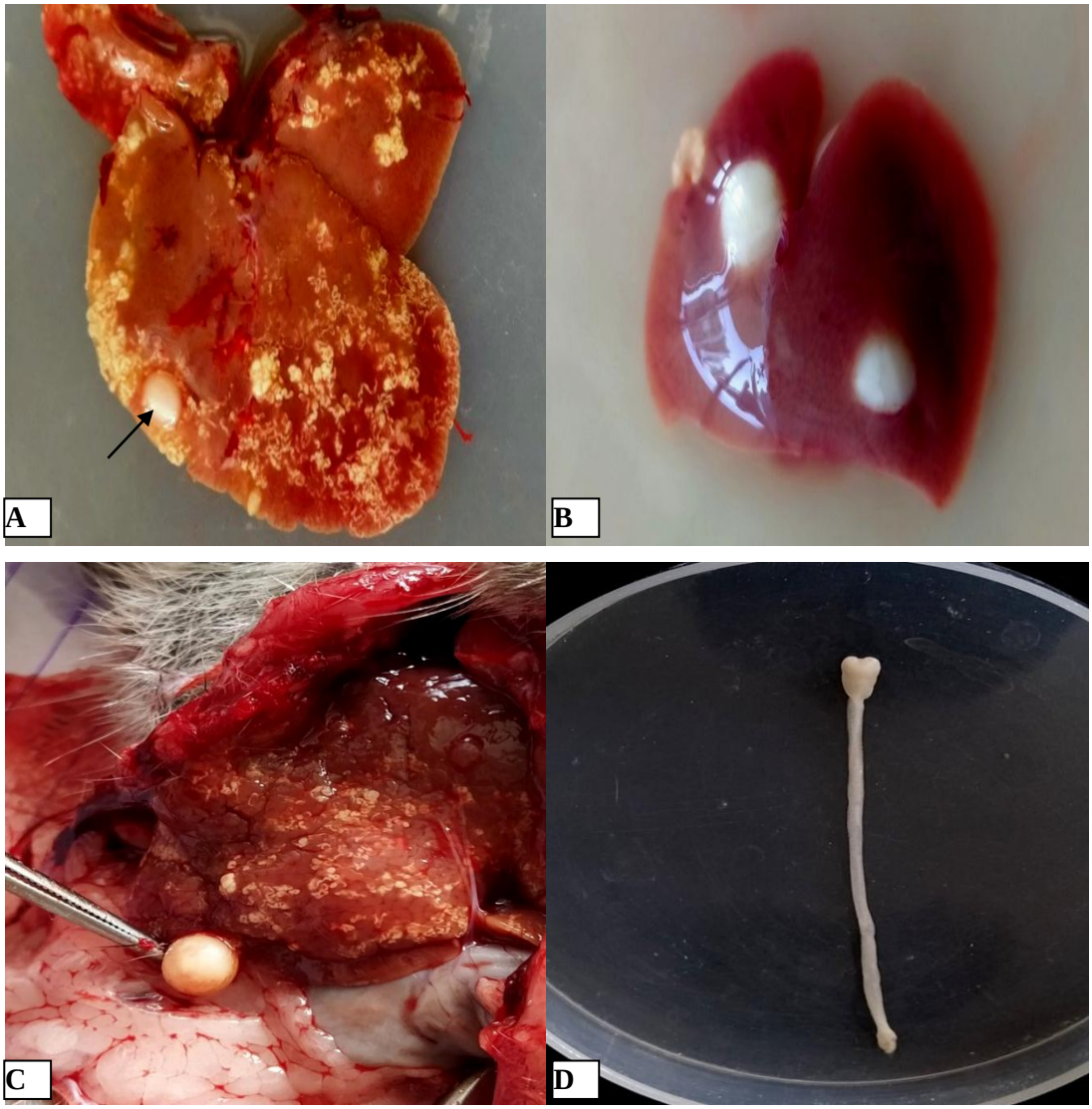

Plate 3. ***Cysticercus fasciolaris*** **found in the liver of** ***Bandicota bengalensis*** **in the form of whitish cysts, A. Concurrent infection of** ***C. fasciolaris*** **(arrow) and** ***Calodium hepticum*** **(yellow lesions), B. Liver with more than one cyst, C. Liver with mature cyst of** ***C. fasciolaris*** **and concurrent infection of** ***C. hepticum*****, and D. Strobilocercus metacestode released from the cyst**

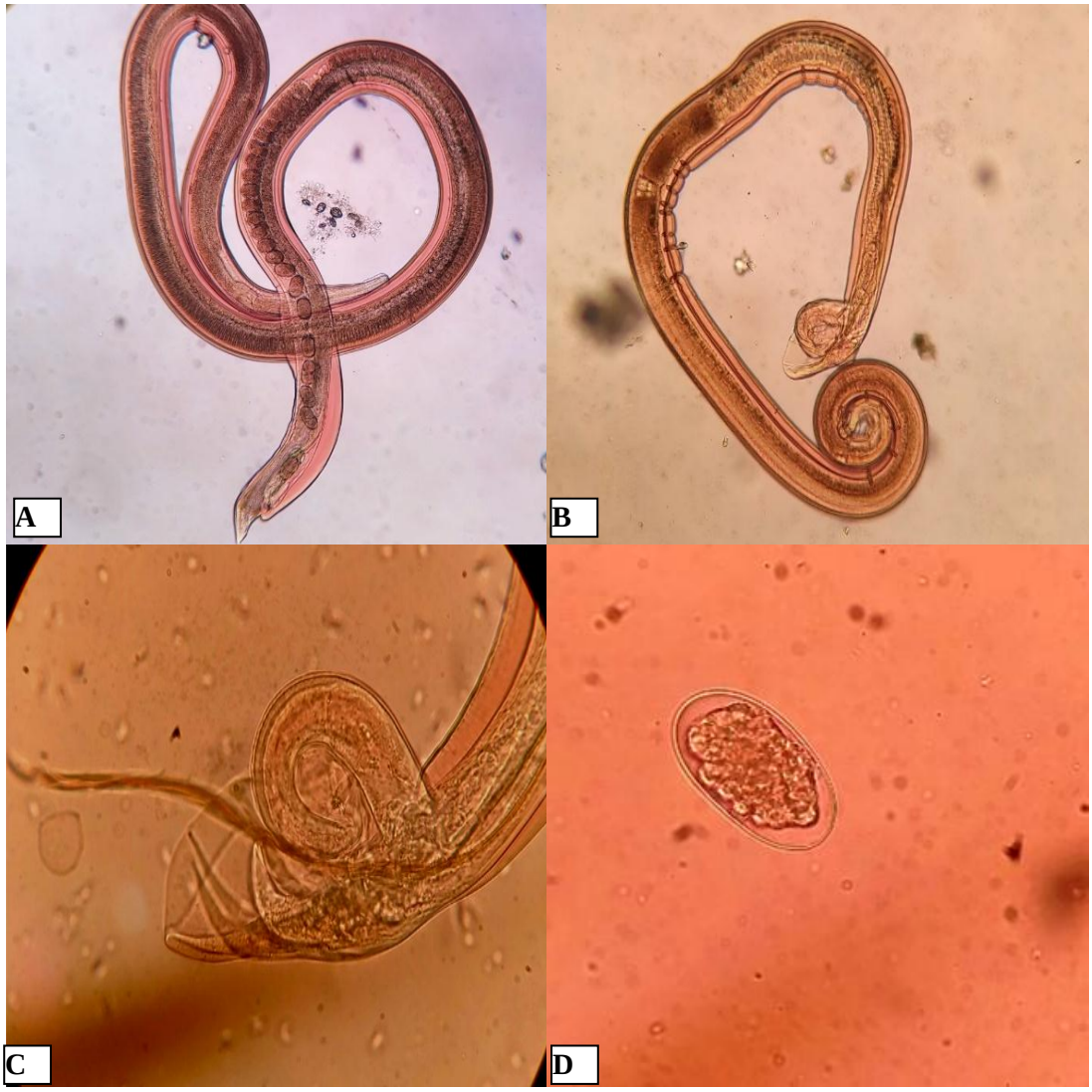

Plate 4. ***Nippostrongylus brasiliensis*** **found in small intestine of** ***Bandicota bengalensis*****, A) Female worm with eggs lined in a row, B) Male worm with copulatory bursa, C) Enlarged copulatory bursa of male worm, and D) Egg found in faecal sample at 400x magnification**

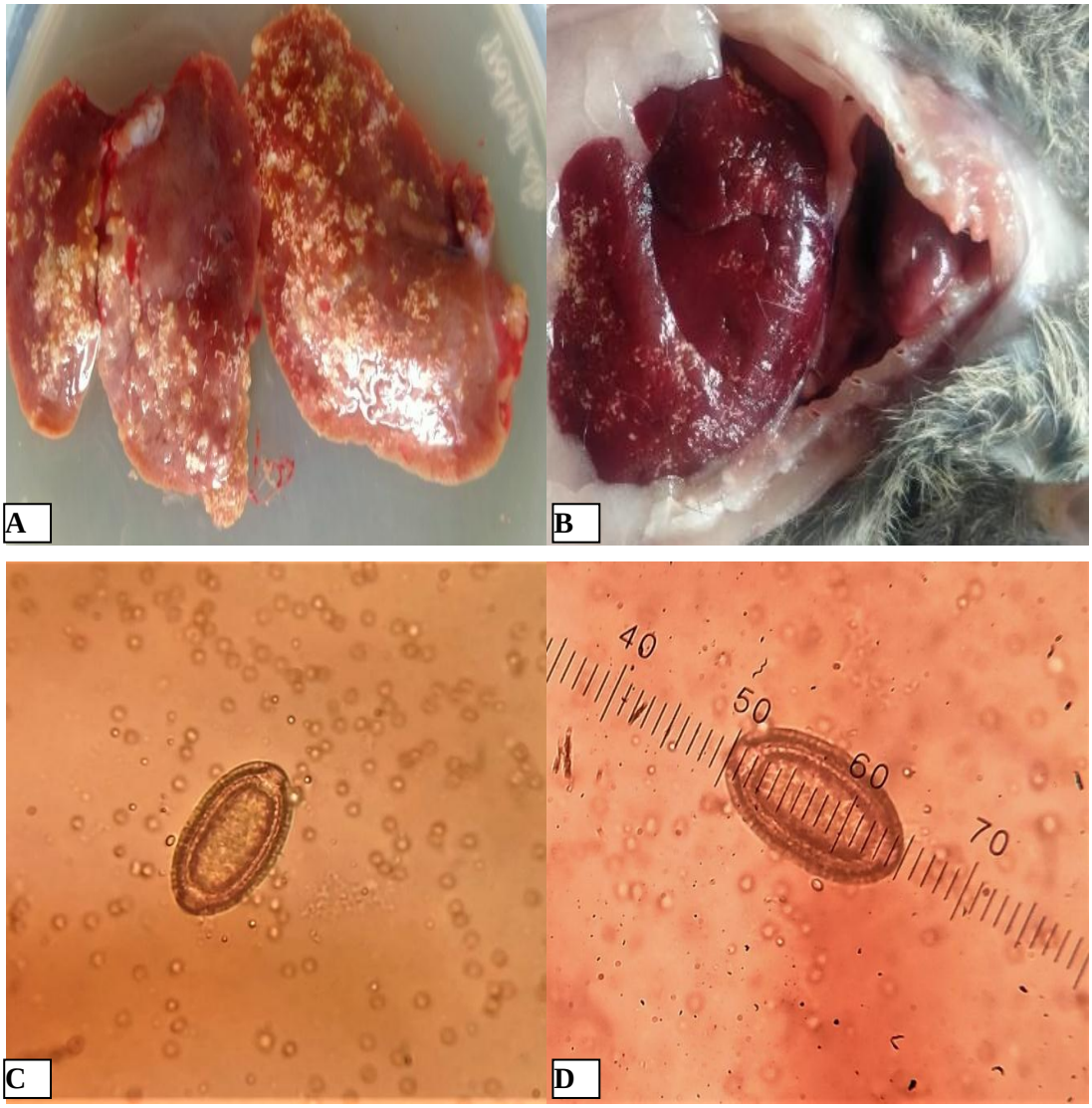

Plate 5. ***Calodium hepaticum*** **infection found in the liver of** ***Bandicota bengalensis*** **in the form of yellowish white lesions or streaks, A) Severe infection, B) Mild infection, C) and D) Bipolar eggs found in liver parenchyma at 400x magnification**

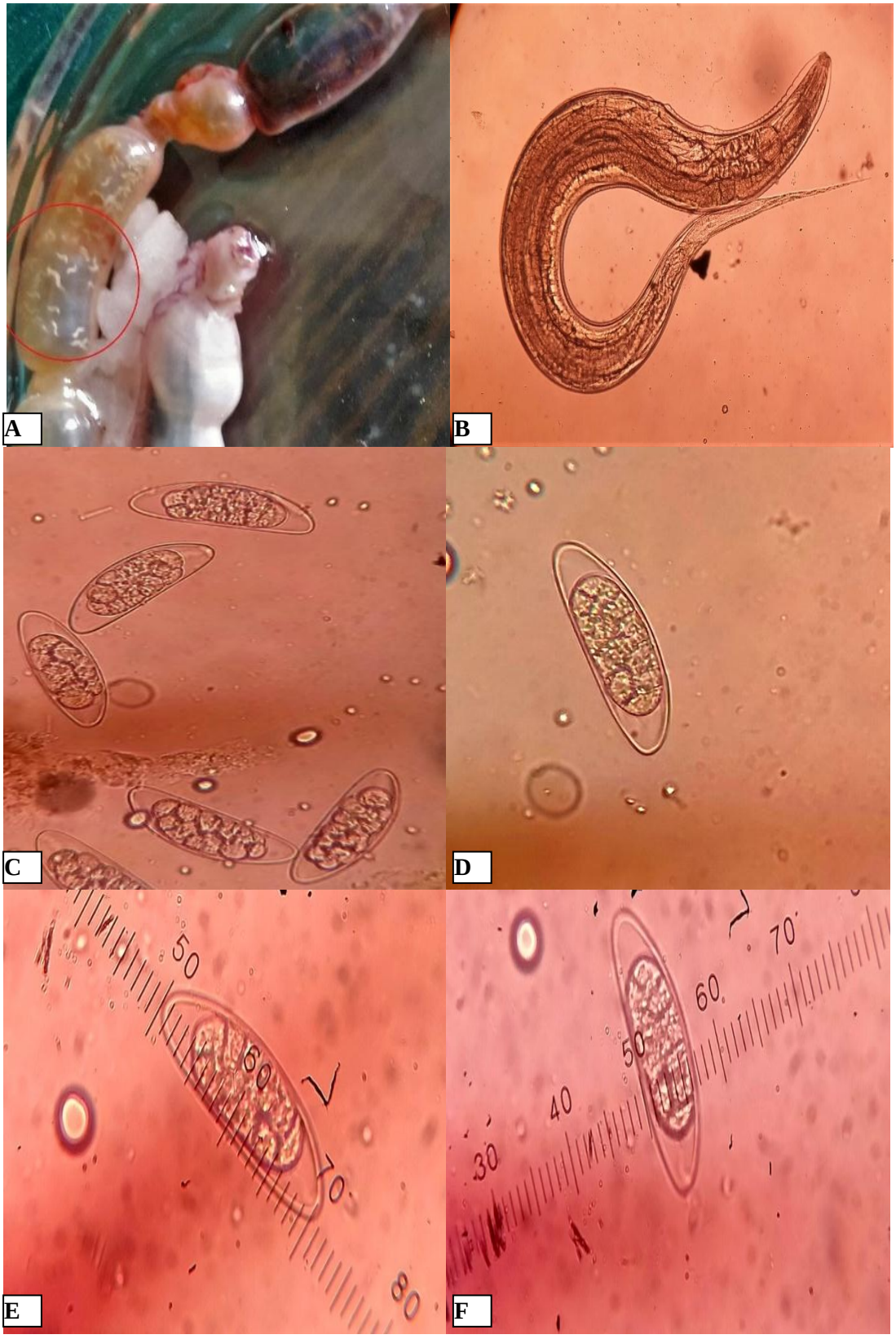

Plate 6. ***Syphacia muris*** **found in large intestine of** ***Bandicota bengalensis*****, A) Worms seen inside the colon, B) Adult** ***S. muris*** **worm, C) and D, Eggs found in faecal sample, and E) and F) Showing dimensions of an egg at 400x magnification**

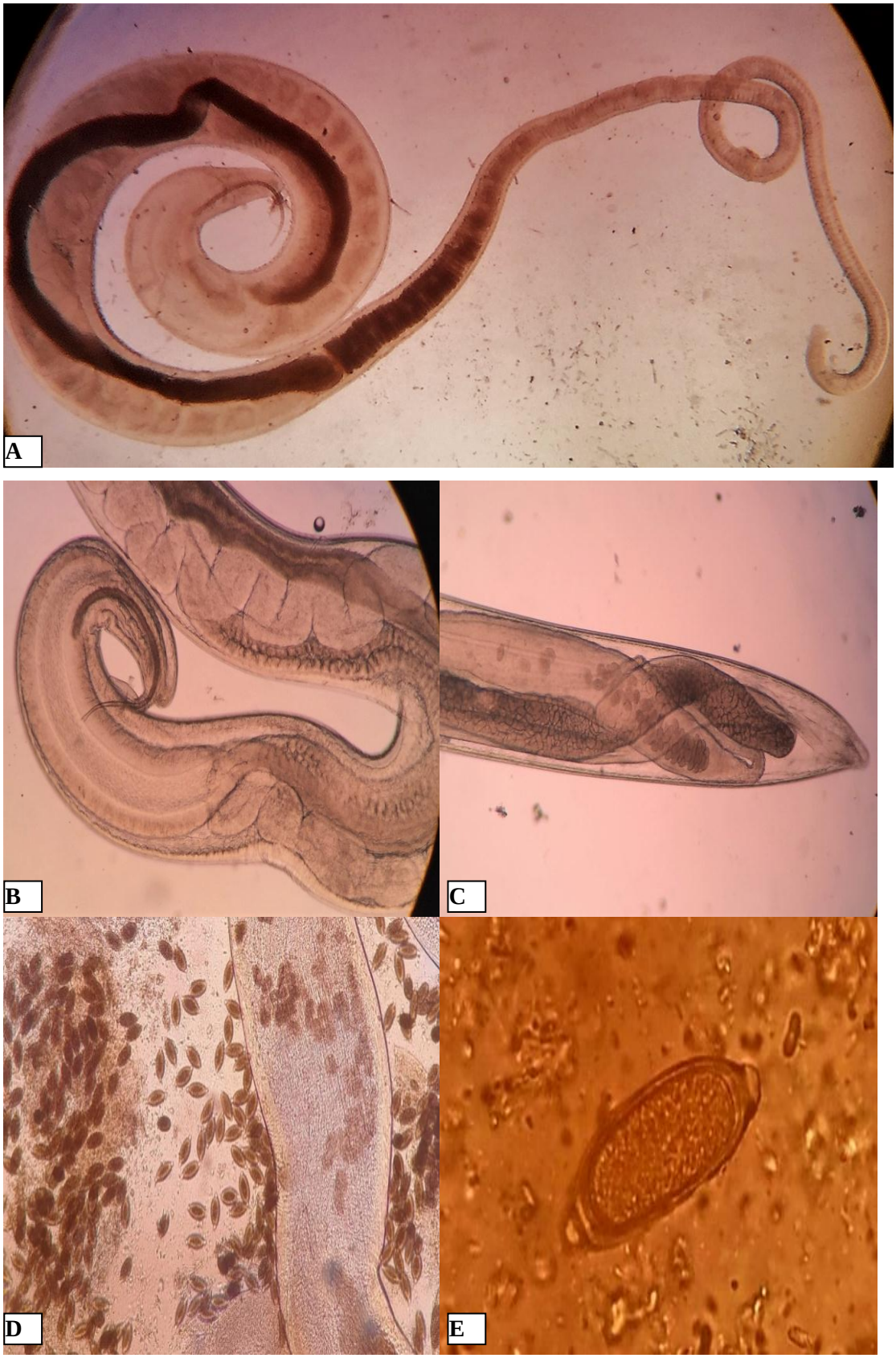

Plate 7. ***Trichuris muris*** **found in caecum of** ***Bandicota bengalensis*****, A) Full adult worm, B) Male worm showing coiled posterior end, C) Female worm showing straight posterior end, D) Eggs released from female worm, and E) Egg with bipolar plugs at both ends at 400x magnification**

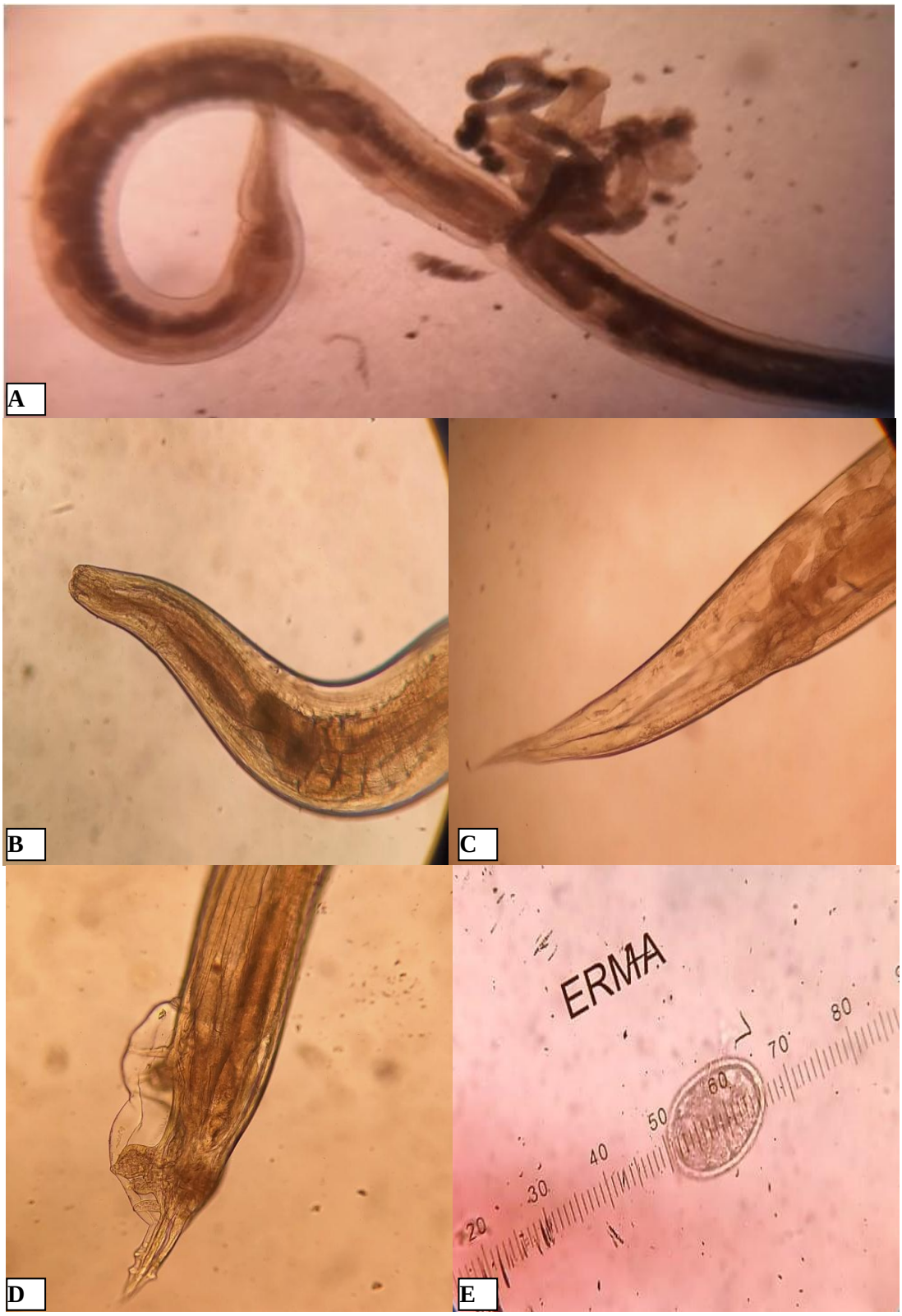

Plate 8. ***Heterakis spumosa*** **found in large intestine of** ***Bandicota bengalensis*****, A) Full adult worm, B) Anterior end of the worm, C) Female worm showing pointed posterior end, D) Male worm showing copulatory bursa at posterior end, and E) Thick walled egg found in faecal sample at 400x magnification**

CHAPTER V

SUMMARY

Rodents living in close association with mankind and its domestic animals serve as a serious threat for transmission of diseases. They are considered as the reservoir hosts for at least 60 zoonotic diseases and play an important role in their transmission directly and indirectly. Direct transmission of diseases is through rat bite, consumption of food or water contaminated with faeces or urine of infected rodent or through inhaling air borne pathogens from rodent excrements. Indirect transmission is through contact with arthropod vectors like ticks, mites, fleas, lice, flies etc. Increase in rodent population can ultimately lead to increased chances of spread of zoonotic diseases resulting in socioeconomic and public health problems.

The lesser bandicoot rat, *Bandicota bengalensis* Grey and Hardwicke is found in both agricultural and commensal situations in Punjab State. It is capable of adapting to all the environmental conditions and has a high reproductive rate. It acts as a reservoir of a number of parasitic infections thereby acting as a major source of zoonotic diseases. Knowledge of rodent parasites is of prime importance in controlling rodent parasite-borne zoonotic diseases in the region. Systematic studies on assessing the potential of *B. bengalensis* inhabiting commensal areas in transmitting parasitic zoonotic diseases in Punjab State are limited. Present study was hence proposed with following objectives:

i) Prevalence of ectoparasites of zoonotic importance on *B. bengalensis*

ii) Prevalence of endoparasites of zoonotic importance in *B. bengalensis*

The *B. bengalensis* examined in the present study for the presence of ecto- and endoparasites of zoonotic importance were collected from fish market and railway station at Ludhiana, Punjab, India from November, 2020 to October, 2021. Total 100 *B. bengalensis* of both sexes were live trapped from the above-mentioned study areas (50 from each location) using multi catch live traps. After noting their sex, weight and maturity status in the laboratory, rats were kept in individual cages with food and water provided *ad libitum*. During examination for ectoparasites, the rats were given mild anaesthesia and their fur, limbs, axillary region, snout and ears were combed over a white tray to dislodge the ectoparasites if any. The ectoparasites fallen in the tray were counted, and preserved in 70% ethyl alcohol for further identification.

Before examining the rats for the presence of endoparasites, their fresh faeces were processed by floatation and sedimentation methods for the presence of eggs/oocysts of different parasites. The eggs found were photographed, measures for their dimensions and. identified using keys. The rats were then dissected through a mid-ventral incision to expose the visceral organs, which were then examined macroscopically for the presence of

endoparasites. Different organs such as liver, stomach, small intestine and large intestine were dissected out and transferred into the petri dishes containing normal saline solution. The adult worms seen through the naked eye were counted, photographed and preserved in 70% ethyl alcohol for further identification. Percent host infected, percent parasite prevalence, parasite intensity and parasite index were calculated. The risk associated with different parasites in relation to different seasons, host age and sex was analysed using Pearson's chi-square test at 5% level of significance.

Total 50 rats comprising 33 females (17 young and 16 adult) and 17 males (13 young and 4 adult) were collected in winter (27), summer (11) and monsoon (12) seasons from fish market, Ludhiana. Total 50 rats comprising 27 females (11 young and 16 adult) and 23 males (11 young and 12 adult) were collected in winter (12), summer (13) and monsoon (25) seasons from railway station, Ludhiana in a period of one year. 28.00% (14) of rats collected from fish market were found infected with only one species of ectoparasites, i.e., Oriental rat flea, *Xenopsylla cheopis*. The infection was highest in adult (35.00%) and female (30.30%) rats in winter season (33.33%). Total 18 rat flea specimens were collected. The parasite prevalence was also high in adult (55.56%) and female (66.67%) rats in winter season (66.67%). The parasite index, an indicator of risk factor analysis was also high in adult (0.50) and female (0.36) rats in winter (0.44) season indicating their involvement in disease transmission. The parasite intensity was, however, high in adult (1.43) and male (1.50) rats in winter and summer (1.33) seasons. Mean parasite intensity and parasite index were 1.29 and 0.36, respectively. 22.00% (11) of rats collected from railway station were also found infected with only one species of ectoparasites, i.e., *X. cheopis*. The infection was highest in young (22.73%) and female (33.33%) rats in winter season (33.33%). Total 16 rat flea specimens were collected. The parasite prevalence was same in both young and adult (50.00%) and female (81.25%) rats in summer season (43.75%). The parasite intensity was, however, high in young (1.60) and male (1.50) rats in summer (1.75) season, while the parasite index was high in young (0.36) and female (0.48) rats in summer (0.54) season indicating their involvement in disease transmission. Mean parasite intensity and parasite index were 1.45 and 0.32, respectively.

Overall, 25.00% rats were found infected with rat fleas from both the locations. Total 34 rat fleas of both sexes were collected from 25 rats with mean parasite intensity of 1.36 and mean parasite index of 0.34. The overall flea index was found to be <1.0 (0.34) indicating low risk of disease transmission. The host age, sex and season had no significant effect on prevalence of flea infestation at both the locations. Female rat fleas had a characteristic spermatheca, while both the sexes had a pygidium at the upper posterior end. Body was segmented and posterior legs were long.

Rats collected from both the locations were found infected with total seven species of

endoparasites, comprising two species of cestodes i.e., *Hymenolepis nana* and *Cysticercus fasciolaris* and five species of nematodes i.e., *Nippostrongylus brasiliensis, Calodium hepaticum, Syphacia muris, Trichuris muris* and *Heterakis spumosa*. The *H. nana* was found in the small intestine and *C. fasciolaris* was found in the liver in the form of whitish cysts. The nematode parasites were found at multiple locations in the host like *C. hepaticum* was found in liver. Infection was identified based on yellowish white streaks or spots on the surface of liver. *N. brasiliensis* was found in small intestine, *S. muris* and *H. spumosa* were found in large intestine and *T. Muris* was found specifically in caecum. Rats were found infected with one or more number of parasite species simultaneously indicating concurrent infection.

Out of total 50 rats collected from fish market and examined for the presence of endoparasites, 86.00% (43) were found infected with one or more species of endoparasites. All the total 27 rats (100.00%) collected in winter season were found infected, while 63.63% and 75.00% rats were found infected in summer and monsoon seasons, respectively. 81.82% female and 94.12% male rats, and 86.67% young and 85.00% adult rats were found infected with endoparasites. This predicted higher rate of endoparasitic infestation and relative risk of disease transmission in young and male rats in winter season.

Among rats collected from fish market, 34.00% were infected with *H. nana*, 12.00% with *C. fasciolaris*, 48.00% with *N. brasiliensis*, 46.00% with *C. hepaticum*, 14.00% with *S. muris*, 26.00% with *T. muris* and 20.00% with *H. spumosa*. Total 42.00% rats were found infected with two species of cestodes and total 123 numbers of parasites were recovered from 21 rats with mean parasite intensity and parasite index of 5.85 and 2.46, respectively. Total 72.00% rats were found infected with five species of nematodes and total 1469 numbers of parasites were recovered from 36 rats with mean parasite intensity and parasite index of 40.80 and 29.38, respectively. In overall, 1592 numbers of parasites (except *C. hepaticum*) were recovered from 43 rats. Prevalence of infection was more in young (66.33%) and female rats (62.00%) in winter season (55.21%). Overall mean parasite intensity and parasite index were 37.02 and 31.84, respectively.

Out of total 50 rats collected from railway station and examined for the presence of endoparasites, 74.00% (37) were found infected with one or more species of endoparasites. 91.67% rats collected in winter season were found infected, while 84.62% and 60.00% rats were found infected in summer and monsoon seasons, respectively. 81.48% female and 65.22% male rats, and 86.36% young and 64.28% adult rats were found infected with endoparasites. This predicted higher rate of endoparasitic infestation and relative risk of disease transmission in young and male rats in winter season.

Among rats collected from railway station, 24.00% were infected with *H. nana*, 20.00% with *C. fasciolaris*, 38.00% with *N. brasiliensis*, 46.00% with *C. hepaticum*, 12.00%

with *S. muris*, 10.00% with *T. muris* and 30.00% with *H. spumosa*. Total 38.00% rats were found infected with two species of cestodes and total 60 numbers of parasites were recovered from 19 rats with mean parasite intensity and parasite index of 3.15 and 1.20, respectively. Total 72.00% rats were found infected with five species of nematodes and total 776 numbers of parasites were recovered from 36 rats with mean parasite intensity and parasite index of 21.55 and 15.52, respectively. In overall, 836 numbers of parasites (except *C. hepaticum*) were recovered from 36 rats. Prevalence of infection was more in young (56.22%) and female rats (70.93%) in monsoon season (63.99%). Overall mean parasite intensity and parasite index were 23.22 and 16.72, respectively.

Out of total 100 rats (50 each) collected from two locations, 79.00% were found infected with endoparasites. Total 2428 endoparasites were collected from 79 rats with mean parasite intensity of 30.73 and mean parasite index of 24.28. The host age, sex and season had no significant effect on prevalence of different endoparasites except *H. nana* and *S. muris* whose prevalence in rats collected from fish market was significantly affected by season.

Macro and microscopic examination of *H. nana* revealed tapeworms to bedorso-ventrally flattened, 2-4 cm long having scolex with four suckers and a retractable rostellum armed with a crown of 20-30 hooks. The strobila was typical with about 200 segments, reproductive organs and gravid proglottids containing a large number of eggs. Numerous eggs of *H. nana* were found in faecal samples. The eggs were oval, small, with oncosphere having six hooks. Hexacanth embryo within the oncosphere contained 4-8 polar filaments. Mostly one cyst (4-12 mm in diameter) of *C. fasciolaris* was found in each animal except one rat in which more than one cyst were found. Each cyst contained a single, live, characteristic strobilocercus larva (10-18 cm in length). The metacestode had armed scolex with two rows of hooks and lateral suckers.

N. brasiliensis was found to inhabit small intestine of *B. bengalensis*. Both male and female parasites of *N. brasiliensis* were recovered. It showed sexual dimorphism as males had characteristic bursa at the posterior end and female ovary had eggs lying in a single row. Gross lesions comprising irregular pale cystic areas were found randomly scattered on the liver surface of rodents infected with *C. hepaticum*. No adult worms were found in the liver. Large deposition of typical eggs with bipolar caps was found scattered in the parenchyma of the liver tissue of rats infected with *C. hepaticum*.

The adult worm of *S. muris* was cylindrical, with rounded anterior end and tapered posterior end. The mouth was surrounded by three distinct lips. Length of adult worm varied from 1.2-3.4 mm. The male worm had a single, long, prominent spicule at the posterior region, while the female worm was long with its vulva in the anterior quarter of the body. Eggs were asymmetrical and slightly flattened on one side and pointed at both the sides. The adult worms of *T. muris* were small having cylindrical non-segmented body. Adult males

were 30-45 mm long with coiled posterior end while the adult females were 35-50 mm long with straight posterior end. Both sexes had a long, whip-like anterior end. A number of eggs of *T. muris* were recovered from the faeces of infected rats. The eggs were with thick brown shell and characteristic bipolar plugs at both the ends. Both male and female adult parasites of *H. spumosa* were found in present study. Worms were about 1cm long, slender in shape. A number of eggs were recovered from the faeces of rats. Eggs were thick-walled.

Despite heavy infection with intestinal parasites (such as *N. brasiliensis, S. muris* and *H. spumosa*) and marked hepatic tissue damage due to severe infection of *C. hepaticum* and *C. fasciolaris* infection, all rats appeared healthy and active suggesting a well-established host-parasite relationship. In view of the diversity and zoonotic nature of rat parasites and the conditions prevailing in commensal habitats where *B. bengalensis* survive and proliferate, they can readily facilitate parasite transmission to humans and other susceptible animals.

The present study indicates *B. bengalensis* inhabiting commensal areas to be risk associated as being loaded with number of ecto- and endoparasites. The close association of these rats to human dwellings may facilitate easy transmission of these zoonotic parasites. Being nocturnal, the rats are very active during night hours, wandering here and there and contaminating the environment, food and water thus responsible for spread of diseases of veterinary and zoonotic importance. Present study suggests that proper rodent pest and vector management should be conducted in animal and human inhabitations to avoid the spread of zoonotic diseases caused by them.

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