Journal of Animal Research: v.8 n.4, p. 667-671. August 2018

DOI: 10.30954/2277-940X.08.2018.18

A Study on Occurrence of *Aspiculuris tetraptera* Infection and Mortality in Mice

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Received: 14 June, 2018 **Revised:** 18 July, 2018 **Accepted:** 21 July, 2018

ABSTRACT

Aspeculuris tetraptera is a pinworm of laboratory and wild rodents. Mice colonies transported from Bombay to Nagpur, showed stress with sudden mortalities of 25 (20%) mice out of total 125, without any further clinical signs. All animals were subjected ~~for~~ to the fecal examination and revealed pinworm infection. It was diagnosed by presence of ellipsoid eggs in the faecal matter of rodents. However, infestation was characterized by rectal prolapse, intestinal impaction, and mucoid enteritis. Intestinal samples especially ceacum and colon were taken out after post-mortem and washed thoroughly with salt water, then the intestines kept in new clean petri plates so as to allow the worms to come out in water in petri plate. After about half an hour, the small worms were collected and were kept in 70% ethanol and later vital statistics was performed. The males worms were 2 mm to 3 mm long and 110 µ to 200 µ wide, with a short conical tail that is 110 µ to 149 µ long. Both spicule and gubernaculum were absent. The females were 3 mm to 5 mm long and 205 µ to 255 µ wide, with a conical tail that is 395 µ to 557 µ long. The eggs are symmetrically ellipsoidal and 70 µ to 100 µ long by 25 µ to 40 µ wide. Control is recommended to remove parasitic eggs from the environment through adequate hygiene to prevent the spread of new infestation.

Keywords: *Aspiculuris tetraptera*, diagnosis, mice, Nagpur.

Pinworms are common parasites in wild and laboratory rodents. The most common pinworm detected in laboratory mice is *Aspiculuris tetraptera*. However, mice infected with *A. tetraptera*, as well as other rodent pinworms (*Syphacia* spp.), are typically symptomless, although rectal prolapse, intestinal impaction, and mucoid enteritis have been associated with severe infestation. Despite their relative nonpathogenicity, pinworm infections induce a Th2-associated immune response.

The prevalence of *A. tetraptera* in wild mouse populations is unknown, however it is likely much higher than in laboratory populations of mice. Susceptibility has been measured in wild-derived mice, as estimated by their parasite burdens, and is highly variable. The prevalence of pinworms in an infected mice colony depends on many factors, including environmental stress, sex, age, strain and immune status of the animal. Male mice tend to suffer more of the parasitic load than female mice, and young mice tend to harbour more than older mice (Taffs, 1975). Laboratory mice tend to be more resistant to infection than wild mice. Athymic nude mice, as might show some susceptibility to the infection.

In mice with normal immune systems, pinworm infections are generally considered mild or non-pathogenic. However, pinworm infection may interfere with research goals in a number of ways. Pinworm infection can increase a mouse's humoral immune response to nonparasitic antigenic stimuli and accelerate the development of the hepatic monooxygenase system.

In athymic nude mice, infection may trigger a lymphoproliferative disorder that eventually leads to lymphoma. *Aspiculuris tetraptera* larvae live in the crypts of Lieberkuhn in the proximal colon, after hatching in the cecum. Unlike *Syphacia* spp., these worms migrate from the proximal to the distal colon to lay eggs. The eggs are excreted in the waste and become infective 5-8 days later. *A. tetraptera* has a 21-25 day prepatent period.

Though pinworm infestation remains common in laboratory rodent colonies, there is little information

regarding current practices for pinworm detection and their relative efficacy.

MATERIALS AND METHODS

The mouse colonies brought from Bombay were kept in 12:12 hour light: dark room. Mice were kept in standard polycarbonate cages, bedded with wooden chip bedding. Cages were covered with wire lids only. Tap water and rodent food were provided to the mice without any restrictions. Cages were changed and sanitized weekly. Due to transportation stress, 25 mortalities were reported suddenly without any clinical signs and immediately after that the rest of the mice were isolated and faecal samples were collected where the mice were diagnosed with pinworm infection. The egg count decreased. The cages were sanitised at weekly intervals and dry bedding was provided regularly. After two weeks the infection was totally decreased and went off with zero eggs in faecal samples.

Samples collected

Feacal samples and intestine specimens were collected immediately after the mice show mortality. Approximately 1 g fecal pellets were collected from fresh faeces. Samples were placed in sterile vials, labelled and analysed within 12 hours. Intestines were kept in sterile petri plates and washed thoroughly to clear the intestines. Intestines were then transferred to new petri plates filled with water so as the worms would come out in water and can be collected.

Faecal egg counts

Faecal egg counts (eggs per g) were performed by a clinical parasitologist who was blinded in regard to mouse species and subspecies. The faecal sedimentation test was used to analyse the samples. Each of the faecal samples was weighed to approximately 1 g, soaked in a few drops of water until soft. The mixture was strained by a tea strainer and poured into a sedimentation flask. Let it stand till the sediment settles at the bottom for atleast 20 minutes. After 20 minutes, discard the whole and take one drop on the clean glass slide from the bottom. Cover the drop with cover slip and then observed under students microscope.

Microscopic study on pinworms

Intestinal samples especially ceacum and colon were taken out after post-mortem and washed thoroughly with salt water, then the intestines are kept in new clean petri plates so as to allow the worms to come out in water in petri plate. After about half an hour, the small worms were collected and were kept in 70% ethanol. A small worm is taken on glass slide to observe under high power microscope.

RESULTS AND DISCUSSION

The faecal examination by simple floatation technique, revealed the numerous eggs of *A. tetraptera* (Fig. 1). The maximum number of eggs observed was 1000 eggs per gram.

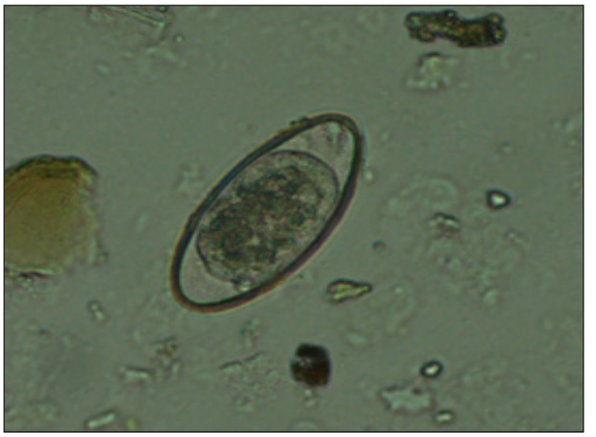

Fig. 1: Egg of *A. tetraptera* in the faecal samples

Traditionally, pinworms are diagnosed in live mice by detecting eggs by means of the anal tape test (SO) (Baker, 2007; Eguíluz *et al.*, 2001; Hill *et al.*, 2009; Pritchett, 2007; Taffs, 1976) and faecal concentration methods (AT) (Baker, 2007; Taffs, 1976; Pritchett, 2007). Faecal concentration techniques, including flotation and sedimentation methods, improve the recovery and identification of parasites (Smith *et al.*, 2007).

Out of 125 mice, 25 (20%) were found dead without showing any further clinical signs and then subjected for post-mortem examination

During post-mortem, cecal and colon contents were collected and observed under microscope and it was observed that majority of the worms were males and females

were very less in number. The direct evaluation of cecal and colonic contents at necropsy is generally considered to be the 'gold standard' for detecting pinworms (Dole *et al.*, 2011; Farrar *et al.*, 1994; Feldman and Bowman, 2007; Ooi *et al.*, 1994). In our study the males were 2 mm to 3 mm long and 110 µto 200 µ wide, with a short conical tail that is 110 µto 149 µ long. Both spicule and gubernaculum were absent. The females were 3 mm to 5 mm long and 205 µ to 255 µ wide, with a conical tail that is 395 µ to 557 µ long. The eggs are symmetrically ellipsoidal and 70 µ to 100 µ long by 25 µ to 40 µ wide. According to external morphological characters it was found to be *Aspiculuris tetraptera* Schulz, 1924. The anterior end has prominent and broad, ending abruptly behind level of oesophageal bulb (Fig. 2). The medial portion shows eggs in intestine (Fig. 3). The distal end has no spicule and gubernaculum (Fig. 4).

Fig. 2. Anterior end of *A. tetraptera*

Fig. 3: Medial portion showing eggs inside uterus

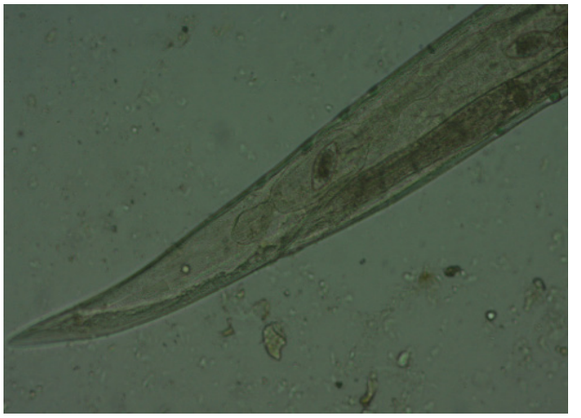

Fig. 4: Distal end of *A. tetraptera*

Aspeculuris tetraptera is very difficult to control as lab animals can get the infection through environment, through transportation stress. A recent study has been characterized *Aspiculuris tetraptera* in India (Goswami *et al.*, 2015).

Antemortem pinworm detection can be challenging, given that testing can produce false-negative results due to testing during the prepatent period or intermittent egg shedding (Bunte and Nolan, 2006; Clarke and Perdue, 2004; Clifford and Watson, 2008; Roble *et al.*, 2012).

Traditional antemortem testing methods generally are considered to be less sensitive than are postmortem testing methods (Clifford and Watson, 2008).

In addition, pinworm detection can be affected by extrinsic factors including worm burden and the ability to transmit infections to soiled-bedding sentinels, which can be influenced further by the parasite's life cycle, including time to egg embryonation (Clifford and Watson, 2008; Effler *et al.*, 2008). Immune resistance can reduce both worm and egg burdens and has been reported regarding both pinworm species (Behnke, 1975; 1976; Clarke and Perdue, 2004).

The incidence of pinworm infection is very common among rodents. These are nematodes from family Oxyuridae having simple and direct life cycle. They are frequent contaminants of both specific pathogen free (SPF) and conventional colonies of laboratory mice. Two species of pinworms that commonly infect laboratory mice are *Syphacia obvelata* and *Aspiculuris tetraptera*. But here we specifically talk about *Aspiculuris tetraptera*. During

their initial period of research, out of those 125 mice, 25 mortalities were reported without any clinical signs.

Aspiculuris eggs are susceptible to heat but highly resistant to cold, desiccation and disinfectants (Oldham, 1967). Peracetic acid (2%), widely used as a bactericide and virucide in work with germ-free animals, but does not kill more than a small percentage of worm eggs (Van der Gulden and Van Erp, 1972). Control is recommended to remove parasitic eggs from the environment through adequate hygiene to prevent the introduction of new infection. Pinworm eggs have been found on equipment, in dust and in ventilation air-intake ducts as reported by Hoag (1961) so thorough cleaning and sterilization of cages and rooms are necessary.

CONCLUSION

After long transportation, sudden mortality was noticed in mice without showing any clinical signs in which 25 mice died out of 125 mice. In conclusion, our study demonstrates increased susceptibility in rodents to pinworm infection during transportation. The most common cause of mortalities is due to transportation stress and change in climatic conditions. Timely cleaning and disinfecting the rooms and cages can prevent the infection to a large extent. If mice are subjected to long distance transportation, they must be first kept isolated for at least 7-10 days for acclimatization and to check further spread of infestation.

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