Ground beetle, *Opatroides frater* (Coleoptera) as natural intermediate host for the poultry tapeworm, *Raillietina cesticillus*

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Abstract Poultry farms in and around Namakkal with a history of tapeworm infection were surveyed for the presence of beetles which could act as intermediate host for the tapeworms. Beetles collected from different poultry farms with suspected tapeworm infection were examined for the presence of metacystode stage of the parasite. A total of 1,880 beetles were collected from 12 poultry farms with suspected tapeworm infection to study the vector potentiality. Out of these, 205 beetles (10.9 %) from nine farms were found to harbour cysticercoids. The percentage of cysticercoid infection in beetles was 8.24, 10.34 and 16.66 % respectively in three different surveys. The beetles harbouring the cysticercoids were identified as *Opatroides frater*, which may be a natural intermediate host for *Raillietina cesticillus*. Infection free young chicks (4 weeks old) were experimentally infected with specific number of cysticercoids and prepatent period of tapeworms was found to be between 12 and 13 days. Gravid segments were expelled between 3 and 4 p.m. consistently. The results of this study would help to formulate suitable control measures against the above tapeworm infection.

Keywords Beetle · *Opatroides fater* · Poultry tapeworm · *Raillietina cesticillus*

Introduction

In India, poultry rearing has become an extremely popular enterprise on account of its rapidly increasing demand. Owing to modern trend of intensive rearing system and use of superior germplasm to produce high yielding strains, chickens have become more susceptible to several pathogens including a number of parasites.

Among parasites of poultry, tapeworms are very common and a recurrent problem (Bhowmik et al. 1982). The distribution and intensity of tapeworms are found to be fairly high in the intensive rearing of chickens and these tapeworms are transmitted by different kinds of invertebrate hosts in and around poultry litter (Harikrishnan and Ponnudurai 2010). The continuous presence of these intermediate hosts account for the persistence of tapeworm infection in chickens. Therefore, effective control of tapeworm infection can only be possible through control of intermediate hosts rather than indiscriminate use of anthelmintics. Investigations into the biology of tapeworm and its intermediate host will be of much significance in formulating suitable control measures.

Materials and methods

Beetles were collected from 12 poultry farms around Namakkal area with a history of tapeworm infection in three different surveys of 850 beetles from nine farms, 580 from two farms and 450 from one farm for 1 year. They were placed in a test tube containing chloroform to facilitate easy handling.

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The beetles were identified based on morphological characters and were confirmed by Department of Apiculture, Agricultural College, University of Agricultural Sciences, Bangalore and Indian Agricultural Research Institute (IARI), New Delhi.

The beetles were examined for the presence of cysticercoids by trimming both the sides of abdomen with a pair of scissors after removing elytra and wings. The incised part of abdomen was teased out gently with the help of a needle in embryo cup containing physiological saline to recover cysticercoids. Mature cysticercoids were visible to naked eyes as white pecks in saline and counted under microscope (250×). Detailed morphological studies were made under binocular microscope (Leitz Labourlux, Switzerland). The average measurements of cysticercoids were undertaken and recorded.

The cysticercoids were stained with methylene blue and identified based on the morphology described by Dutt et al. (1961). Confirmation of the identity was done by feeding the mature cysticercoids to experimental chickens. one month old white Leghorn chicks were experimentally fed with 25 mature cysticercoids each, after 24 h of fasting. The droppings of these birds were regularly monitored at different intervals for the presence of gravid segments as described by Nadakal et al. (1970). The day of first appearance of gravid segments was noted for determination of prepatent period.

The scolex, mature and gravid segments of adult worms were identified by trichrome or acid alum carmine staining. They were flattened by pressing them between two slides and fixed in 10 % formalin for about 24 h and then stained with trichrome stain (Chromotrope 2R-0.39 g, Rhodamine 13-0.19 g, Orange G-0.39 g, Methyl Orange-0.29 g, Fast green-0.15 g, Aniline blue 0.1 g, and phosphotungstic acid-0.35 g) or acid alum carmine stain after thorough washing in tap water to remove traces of formalin. After staining, the specimens were differentiated in 1 % acid alcohol (1 ml of HCL in 99 ml of 70 % alcohol) and then washed in ammonia water (1 or 2 drops of liquid ammonia in 5 ml of water) till traces of acid were removed. They were then dehydrated in ascending grades of alcohol such as 70, 90 % and absolute alcohol and cleared in creosote. Permanent mounts were prepared in DPX or Canada balsam for detailed examination.

Results

A total of 1,880 beetles were collected from 12 farms in three different surveys. Out of these, 205 (10.9 %) beetles from nine farms were found to harbour cysticercoids of *Raillietina cesticillus*. The number of farms surveyed, the percentage of cysticercoid infection in beetles and their intensity are summarized in Table 1.

The beetles harbouring the cysticercoids of *R. cesticillus* were identified as *Opatroides frater* (Fig. 1) by Department of Apiculture, Agricultural College, University of Agricultural Sciences, Bangalore and Indian Agricultural Research Institute (IARI), New Delhi.

Based on the morphology and morphometry, the cysticercoids were identified as *R. cesticillus* (Fig. 2). The mature cysticercoids were oval in shape with an average diameter of $371 \times 281 \mu\text{m}$. They had an invaginated scolex at the broader end and the point of invagination was marked by a deep groove. The rostellum had two rows of hooks with unarmed suckers. The calcareous corpuscles were distributed uniformly within the cyst.

Twenty five mature cysticercoids were fed to each experimental chick and the droppings were examined daily for the presence of gravid segments and the prepatent period was found to be between 12 and 13 days post infection. A definite periodicity in the expulsion of gravid segments was observed between 3 and 4 p.m. consistently. The gravid segments appeared as pearly white wriggling on

Fig. 1 Dorsal view of *O. frater*

Table 1 Vector studies on beetles collected from poultry farms

No. of trials	Number of farms surveyed	Number of farms positive	Percentage of cysticercoids in beetles	Intensity of cysticercoids per beetle				
				0–10	11–50	51–100	101 and above	Maximum
1	9	6	8.24 %(70/850)	1	8	3	2	485
2	2	2	10.34 %(60/580)	5	11	4	2	320
3	1	1	16.66 %(75/450)	4	14	8	3	276

Fig. 2 Cysticercoid of *R. cesticillus* ($\times 250$)

Fig. 5 Mature segments of *R. cesticillus* ($\times 250$) (Acid alum carmine stain)

Fig. 3 Intestine with *R. cesticillus*

Fig. 6 Egg of *R. cesticillus* ($\times 250$)

Fig. 4 Scolex of *R. cesticillus* ($\times 250$) (Trichrome stain)

the surface of droppings and resembled a sago granule. An average of 17 worms were observed in birds fed with 25 numbers of cysticercoids (Fig 3).

The tapeworms recovered from experimental infection were identified as *R. cesticillus* based on the morphological

characters. The worms measured 6–17 cm $\times$ 1–2 mm. The scolex was robust with wide rostellum, armed with double row of heavily armed hammer shaped hooks and with four suckers which were unarmed, circular and inconspicuous (Fig. 4). In mature segments, genital pores alternated irregularly, located anterior to middle of segment margin. Ovary and vitellaria were present (Fig. 5). Gravid segments contained numerous egg capsules and each revealed a single egg. The eggs measured 86 $\times$ 72 μ m in diameter. The second and fourth membranes were connected by two small filamentous structures which were attached to the fourth membrane inward in a funnel like fashion. Three pairs of embryonated hooks were present (Fig. 6).

Discussion

Beetles observed in the present study were identified as *O. frater*, a tenebrionid beetle. In an earlier study at Namakkal, although the beetle was identified as a tenebrionid,

its specific identity remained to be confirmed (Ponnudurai and Chellappa, 2001). Presently, beetles collected from poultry farms were infected with one species only, *R. cesticillus* as against mixed infection comprising of both *R. cesticillus* and *Choanotaenia infundibulum* in an earlier survey (Ponnudurai and Chellappa, 2001). Earlier, Dutt and Sinha (1961) incriminated *Anthicus confucii* as natural intermediate host for *R. cesticillus* whereas Ponnudurai and Chellappa (2001) incriminated tenebrionid beetles for both *C. infundibulum* and *R. cesticillus*. In the present survey, the tenebrionid beetle harbouring cysticercoids of *R. cesticillus* was identified as *O. frater*. The identification of *O. frater* harbouring the cysticercoids of *R. cesticillus* is the first record of these beetles as natural vectors at Namakkal area and is a noteworthy observation.

The rate of infection in tenebrionid beetles was 10.9 % in the present survey. Earlier, Ponnudurai and Chellappa (2001) recorded 80 % cysticercoid infection in tenebrionid beetles. The wide variation in the percentage of incidence rate between these studies could be due to the number of farms surveyed, beetles collected and single or mixed infection. However, the major reason for persistence of tapeworm infection in Namakkal area could be due to the abundance of beetles in poultry litter and ineffective control measures taken against them.

The mature cysticercoids of *R. cesticillus* averaged $371 \times 281 \mu\text{m}$ and variation in size were noticed when more number of cysticercoids was present in a single beetle. Similar observation was made by Dutt et al. (1961) who reported variation in size according to the number of cysticercoids present within the beetle with an average of $382 \times 312 \mu\text{m}$. Ponnudurai and Chellappa (2001) recorded an average of $292 \times 212 \mu\text{m}$ for mature cysticercoids. The present observation is in accordance with the above findings.

In the present survey, the maximum number of cysticercoids obtained from a single beetle was 485, whereas Ponnudurai and Chellappa (2001) recorded a maximum of 150 in tenebrionid beetle. Dutt and Sinha (1961) could

recover only a maximum of eight cysticercoids from one beetle.

The results of the life cycle studies in experimental chickens revealed that the prepatent period of *R. cesticillus* was found to be between 12 and 13 days. This is in conformity with the findings of Dutt et al. (1961) who observed the prepatent period of *R. cesticillus* to vary from 13 to 16 days. Ponnudurai and Chellappa (2001) also recorded the prepatent period of *R. cesticillus* to be 14 days. A definite periodicity in the expulsion of gravid segments was noted and majority of the segments were seen in the droppings between 3 and 4 p.m. in the afternoon. This observation is in accordance with the earlier reports of Dutt et al. (1961) and Ponnudurai and Chellappa (2001). The former stated that excretion of gravid segments occurred late mid day around 3 p.m. while the latter reported that the expulsion of gravid segments occurred between noon and 3 p.m.

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