



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(4): 1487-1489

© 2018 JEZS

Received: 02-05-2018

Accepted: 03-06-2018

Niranjana Sahoo

Department of Veterinary
Epidemiology and Preventive
Medicine, College of Veterinary
Science and Animal Husbandry,
Bhubaneswar, Odisha, India

Sonali Sahoo

Department of Veterinary
Epidemiology and Preventive
Medicine, College of Veterinary
Science and Animal Husbandry,
Bhubaneswar, Odisha, India

Bijayendranath Mohanty

Department of Veterinary
Parasitology, College of
Veterinary Science and Animal
Husbandry, Bhubaneswar,
Odisha, India

Hemant Kumar Khuntia

Indian Council of Medical
Research-Regional Medical
Research Centre, Bhubaneswar,
Odisha, India

Bikash Kumar Behera

Centre for Wildlife Health,
College of Veterinary Science and
Animal Husbandry,
Bhubaneswar, Odisha, India

Sourabh Ranjan Hota

Centre for Wildlife Health,
College of Veterinary Science and
Animal Husbandry,
Bhubaneswar, Odisha, India

Correspondence

Niranjana Sahoo

Department of Veterinary
Epidemiology and Preventive
Medicine, College of Veterinary
Science and Animal Husbandry,
Bhubaneswar, Odisha, India

Molecular identification of *Spirometra erinaceieuropaei* infection in a dog with its successful treatment

Niranjana Sahoo, Sonali Sahoo, Bijayendranath Mohanty, Hemant Kumar Khuntia, Bikash Kumar Behera and Sourabh Ranjan Hota

Abstract

Present report deals with molecular diagnosis of *Spirometra erinaceieuropaei* infection in a 10-month-old labrador bitch weighing 28 kg body weight with its successful treatment. The dog was passing white flat worms at 7-10 days interval. Interrogation of owner revealed that the pet had the fascination of eating a particular species of frog identified as *Uperodon taprobanica* found in the premises. Gross examination of tapeworm segments showed centrally located uterine pore measuring an average of 10.0 mm width and 2.0 mm length. There was presence of operculated yellowish-brown coloured eggs pointed at either end in the stool. Molecular test confirmed *Spirometra erinaceieuropaei* infection with a product size of 440 bp. Praziquantel @ 150 mg daily orally for three consecutive days reduced pre-treatment faecal EPG from 1180 to 950 (19.5%), 354 (70.0%) and 0 on 1, 3 and 7-day post treatment, respectively. Excretion of adult parasites in the faeces disappeared upto 90-days post treatment.

Keywords: *Spirometra erinaceieuropaei*, dog, praziquantel, India

1. Introduction

Cestodes belonging to the genus *Spirometra* are occasionally reported in dogs and cats across the globe [1, 2]. Fresh water crustacean of the genus *Cyclops* and amphibians like fish, frog or reptile act as first and second intermediate hosts, respectively. Definitive hosts get infection on consumption of the intermediate host with infective stage of the parasite i.e., plerocercoids or spargana. Ingestion of paratenic hosts like primates, pigs, weasels, rodents, insectivores, and birds could be another possible means of transmission. Disease has been reported from wild animals i.e., wild felids [3], wild-caught baboons [4], cynomolgus macaque [5] and russel's viper snake [6]. Present study records *Spirometra erinaceieuropaei* infection in a labrador dog with its successful treatment in Bhubaneswar, India.

2. History

A 10-month old labrador dog weighing 28 kg from Bhubaneswar, India (20.2961°N, 85.8245°E) was presented in the Veterinary College hospital with complaint of intermittent passing of flat worms at 7-10 days interval inspite of repeated deworming with anthelmintics like praziquantel, albendazole or pyrantel pamoate at two weeks interval commencing from 4-month age either at the advice of field veterinarians or at owner's will. However, such medication failed to cease excretion of white segments. Interrogation of the owner revealed an interesting fact that the pet had the fascination of eating frogs found in the vicinity.

3. Material and methods

On clinical examination, the pet was found apparently healthy except the presence of dandruff on body coat. The faecal sample was subjected to microscopic examination for epg by Mac Master's techniques [7] and morphology was studied after staining with borax carmine. PCR was carried out using proglotids to confirm species. The owner was requested to collect that particular frog alive often ingested by the dog from the house premises.

3.1 Molecular diagnosis through PCR

Genomic DNA of the parasite was extracted using commercially available DNA mini kit (QIAGEN, USA). According to the manufacturer's instructions, 25mg each of the three

different excreted parasite segments were taken for the said purpose. The PCR primers used were p1f (5'-TGGTTTTTTGGACATCCTGAA-3') and p1r (5'-ATCACATAATGAAAGTGAGCC-3')^[8]. PCR was performed in a final reaction volume of 25µl reaction mixtures containing 100-150ng/µl template DNA, 2.5µl 10X PCR buffer, 1.5µl MgCl₂ (25mM), 2µl dNTP mixture (2.5mM), 0.3µl Taq DNA Polymerase(1U) and 1.0µl of each primer (20pM). The reaction mixture was placed on a heating block of a programmable thermocycler. The PCR reaction was performed as follows: 1 cycle of initial denaturation at 94 °C for 3 min, 35 cycles at 94 °C for 20 sec, 46 °C for 40 sec, 72 °C for 1 min, and incubation at 72 °C for 4 min. The amplification products were subjected to electrophoresis on 1.5% agarose gel with ladder and the amplified products were visualized using Gel Documentation system. The purified PCR product was subjected to sequencing followed by BLAST to correlate species.

Pet was administered with 150 mg praziquantel (Tablet Droncit) i.e., close to 5.0 mg/kg b.w. daily for three consecutive days before any access to feed. Owner purchased the prescribed tablet from the local market. Faecal EPG WAS calculated both pre-treatment and 1, 3 and 7 day post-treatment

4. Results and discussion

On an average, each proglotid measured 10.0 mm width and 2.0mm length. Microscopic examination of faecal sample showed operculated yellowish-brown coloured eggs pointed at either ends suggestive of *Spirometra* sp. eggs. Examination of stained parasite in 40 X magnification revealed spiral shaped uterus, a characteristic feature of *Spirometra* sp cestode (Fig 1). A product size of 440 bp visualized under Gel Documentation System was considered positive for *Spirometra erinaceieuropaei* (Fig 2). The sequence was compared using BLAST and was found to have 99% homology with the reference sequence of NCBI database. The morphological features of parasites and their eggs along with molecular test confirmed natural infection of *Spirometra erinaceieuropaei* in the labrador dog. Stray reports of *Spirometra* sp. infection in dogs and cats have been reported from India^[1, 9]. Proliferative sparganosis associated with lodgment of spargana in visceral organs causing chronic inflammation and fibrosis has been reported in dog^[10]. The frog species repeatedly ingested by the pet dog was identified as *Uperodon taprobanica* (Fig 3).

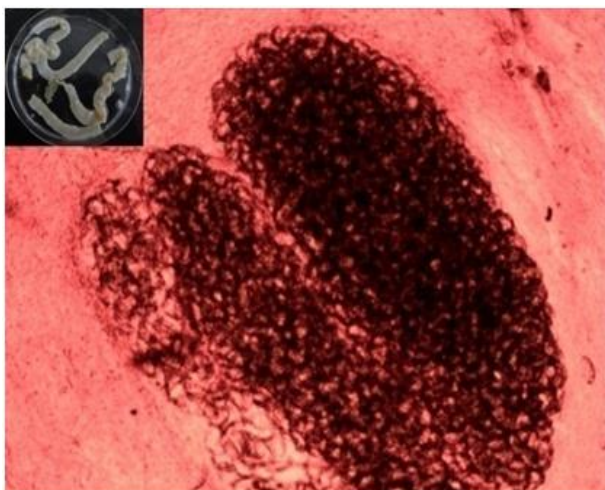


Fig 1: Spiral shaped uterus of *Spirometra erinaceieuropaei*

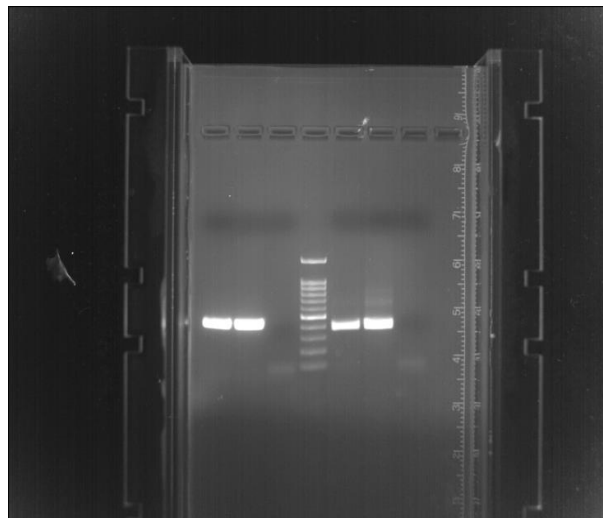


Fig 2: Product size of 440 bp positive for *Spirometra erinaceieuropaei*



Fig 3: *Uperodon taprobanica* recovered from the owner's premises

Spirometra erinaceieuropaei had earlier been detected in African lion, jungle cat, fishing cat, clouded leopard and tiger of Baranga Zoo, Odisha^[3]. These reports were suggestive of the dog being infected by the above species of parasite and urged us to conduct molecular examination for confirmatory diagnosis.

Pre-treatment faecal EPG count of 1180 was reduced by 19.5% on 1st day and 70% on 3rd day post-treatment. Faecal sample was negative for eggs from 7th day onwards. Praziquantel is usually recommended @5.0 mg/kg bw or 50.0mg upto 10 kg body wt as a single dose against cestode infection in dog. But such dose proved non-responsive to present *Spirometra* infection during earlier treatment. Praziquantel @ 7.5mg/kg for two consecutive days was reported to be effective for dogs against *Spirometra* infection^[2]. Praziquantel @ 5.0 mg/kg b.w. daily for three consecutive days was selected for easy calculation of total dose on the basis of its availability, neither eggs nor segments were detected in the faeces upto 90 days following treatment, hence proved efficacious.

Spirometra sp. infection, reported in cats and dogs, is usually asymptomatic in nature. Enteritis and weight loss are seldom reported in individual animals. However, the present pet did not exhibit any of the signs. This could be attributed to repeated administration of anthelmintics that might have

reduced the parasitic burden. The dandruff noticed on the body coat could be correlated with deficiency of micronutrients. This could be corroborated to selective uptake of vitamin B₁₂ by plerocercoid and adult spirometra has been demonstrated [11].

Man gets infection through ingestion of infected cyclops or undercooked intermediate or paratenic host or by application contaminated tissues on skin as poultices. Cases of human sparganoses have been documented involving different organs like eye, brain and spinal cord, inguinal region, draining fluid of perinephric area [2, 12, 13].

Plerocercoids have been detected in various species of frogs across the globe like *Rana tigrina*, *Rana nigromaculata*, *Rana limnocharis*, *Rana guentheri*, *Paa yunnanensis*, *Litoria caerulea*, *Litoria aurea*, *Litoria peronii*, *Litoria gracilentia* [14, 15]. The present case revealed *Uperodon taprobanica* as an intermediate host for the transmission of *Spirometra* sp. Commonly known as Asian painted frog or Sri Lankan Bullfrog, *U. taprobanicus* is a nocturnal, semi-fossorial insectivorous microhylid species. It inhabits in wetlands, riverbanks, forests and residential, agricultural and urban areas. It is a medium sized frog with a tiny head, wide mouth, short snout and squat round body. Hind legs and feet are short and thick with feet having two broad sharp spades used for digging, Digits are long, rather slender without webs and tips are enlarged to form adhesive discs. First finger is shorter than the second one. The species is characterized by the thick, black-edged, light orange band which extends from the eye to the groin along each dorso-lateral side of the body. It is listed as Least Concern under IUCN Red List. The frog is endemic to South Asia (Bangladesh, eastern India and Sri Lanka). When the first assessment of this species was made, it was included under the genus *Kaloula*. Recently, it has been transferred to the genus *Uperodon* [16]. This species has also been recorded from the Karlapat Wildlife Sanctuary of Odisha [17].

The pet owner was advised to prevent ingestion of frogs thereby avoiding re-infection or dissemination of infection to other susceptible hosts. The present report would definitely help the medical community to be vigilant on this zoonotic disease in this part of the globe as the human infection is predominant in Asia.

5. Conclusion

Spirometra erinaceieuropaei infection diagnosed in a 10-month old Labrador pet dog on the basis of morphology of eggs and gravid segment and PCR on proglotids was successfully treated with praziquantel @ 5.0 mg/kg bw for three consecutive days.

6. Acknowledgement

Authors are thankful to the pet owner who cooperated with us during the entire period of investigation.

7. References

1. Saleque A, Juyal PD, Bhatia BB. *Spirometra* sp. in a domestic cat in India. *Vet Parasitol.* 1990; 35:273-6.
2. Little S, Ambrose D. *Spirometra* infection in cats and dogs. *Small Animal/Exotics Compendium.* 2000-22.
3. Patnaik MM, Acharjyo LN. Notes on the helminth parasites of vertebrates in Baranga zoo (Orissa). *Indian Veterinary Journal.* 1970; 47:723-30.
4. Lee MN, Hubbard G, LoVerde P, Carvalho-Queiroz C, Conn DB, Dick Jr EJ *et al* Schlabritz-Loutsevitch N.

5. Sparganosis in wild- caught baboons (*Papio cynocephalus* Anubis). *Journal of Medical Primatology.* 2006-36.
5. Bauch *et al*, Joubert C, Helies JM, Lacour SA, Bosquet N, Le Grand R *et al* Disseminated sparganosis in a cynomolgus macaque (*Macaca fascicularis*). *J Comp Pathol.* 2013; 148:294-7.
6. Kavitha KT, Latha BR, Sundar STB, Sridhar R, Basith SA. Sparganosis in a Russell's viper snake: a case report. *J Parasit Dis.* 2014; 38:394-395.
7. Soulsby E JL, Helminths, Arthropods. Protozoa of Domestic Animals. 7th edition, Elsevier, Reed Elsevier India Private Limited, New Delhi, 1982; 766-771
8. Jeon HK, Park H, Lee D, Choe S, Kim KH, Huh S *et al* Human Infections with *Spirometra decipiens* Plerocercoids Identified by Morphologic and Genetic Analyses in Korea. *Korean J Parasitol.* 2015; 53:299-305
9. Pal MG, Chakrabarti A, Pramanik AK, Pradhan KK, Chatterji A. Spirometrid tapeworm in a mongrel dog. *Indian J Anim Health.* 1981; 20:71-2
10. Drake DA, Carreño AD, Blagburn BL, Little SE, West MD, Hendrix CM, Johnson CM. Proliferative sparganosis in a dog. *J Am Vet Med Assoc.* 2008; 233: 1756-60.
11. Marchiondo AA, Weinstein PP, Mueller JF. Significance of the distribution of co-vitamin B₁₂ in *Spirometra mansonioides* (Cestoidea) during growth and differentiation in mammalian intermediate and definitive hosts. *Int J Parasitol.* 1989; 19:119-124.
12. Nath R, Gogoi RN. Ocular sparganosis from Assam. *Trop Parasitol.* 2015; 5:64-7.
13. Sabu L, Lakshmanan B, Kumar PS. Occurrence of Human Sparganosis in Kerala. *J Parasit Dis.* 2015; 39: 777- 779.
14. Li MW, Song HQ, Li C, Lin HY, Xie WT, Lin RQ *et al* Sparganosis in Mainland China. *International Journal of Infectious Diseases.* 2011; 15:154-156
15. Berger L, Skerratt LF, Zhu XQ, Young S, Speare R. Severe sparganosis in Australian Tree Frogs. *Journal of Wildlife Diseases.* 2009; 45:921-92
16. Aditya G, Hazra N. Occurrence of Sri Lankan bullfrog, *Uperodon taprobanicus* (Parker, 1934) (Amphibia: Anura: Microhylidae) in Burdwan, West Bengal, India. *International Journal of Zoology Studies.* 2016; 5:42-44
17. Deuti K, Raha S. Amphibians of Karlapat and Konarak-Balukhand Wildlife Sanctuaries, Odisha. *Rec. Zool. Surv. India.* 2010; 110:7-26