Experimental Studies on Domestic Cat as a Definitive Host of *Spirometra* species

Abstract

In the present study domestic cats were used to elucidate if they were definitive hosts of *Spirometra* species. Cats were orally fed with spargana recovered from naturally infected frogs and from experimentally infected hamster mice. The spargana fed to domestic cats developed to adult worms. Eggs discharged by the adult worms were collected from faeces of infected domestic cats. Cats were sacrificed, adult worms were recovered, measurements and morphology of the worms were taken. All results were compatible with *Spirometra* species. Therefore, the study confirms that domestic cats are definitive host of *Spirometra* species.

Keywords: Experiment, domestic cat, definitive host, *Spirometra*, spargana

Introduction

The genus *Spirometra* belongs to the order Pseudophyllidea family Diphyllobothriidae. This cestode is generally distributed all over the world [1] Many studies have been carried out on this group of parasites. The spargana (plerocercoid) and adult stage of *Spirometra* utilize a wide range of hosts including man and his domestic animals.In the life cycle of *Spirometra* species there are two intermediate hosts involved before the definitive host where the adult stage of the parasite is observed. The first intermediate host is the *Cyclops*. The second intermediate host are the reptiles, amphibians, birds and mammals [2,3,4,5,6].The definitive host is canines, carnivores and man is an accidental definitive host.Few studies have been carried out to determine if cats are definitive hosts of *Spirometra* species. McIntosh [7] and Mueller [8] showed the presence of *Spirometra* infection in cats, bobcats, dogs and other carnivores. McIntosh [7] studied carnivores and reported adult *Spirometra*worms in the small intestine of the cats, bobcats and dogs. Site of attachment of adult worm in the small intestine of cats has been reported by Mueller [8]. Morphology of adult worm recovered from cat by the previous workers has been reported [8,9]. The aim of this study was to determine if domestic cat was a definitive host of *Spirometra* species.

Materials and method

In this study, a total of 5 domestic cats (Felis sp.) were used to determine if (cats can serve as)weredefinitive host in the life cycle of *Spirometra* species. (5 stray cats of ages ranged from 8-10 months were used in this study). The first batch of 3 cats were infected with spargana recovered from the first batch of hamster mice infected with spargana collected from naturally infected frogs. The other two cats were infected with spargana recovered from the second batch of hamster mice infected with

procercoids. Prior to infection, the cats were de-wormed with TROY Puppy & Kitten worm syrup (Piperazine Citrate). The dosage was 1ml/ Kg BW for three consecutive days. After de-worming, the stool was examined daily (by using faecal smear and sedimentation methods) for 5 days and it wasthey werenegative for any eggs. The cats were observed for another 5 days before being fed with the spargana.

Feeding Spargana to Cats

Each of the three cats were fed with 2 spargana recovered from laboratory infected hamster mice.The procedure for infecting cats with spargana was: A big towel which was folded twice and placed on the table. Cat was taken, placed on the folded towel and wraped up to the level of the neck. All feet were wraped in folded towel to avoid being injured by their sharp claws. Cat was held firmly with hands around the neck. A 5 ml syringe with scoleces of spargana suspended in 2 ml of Normal Saline was introduced into the mouth of the catand contents of the syringe were forced fed. Cats were housed in the Animal House, Institute of Biological Sciences, University Malaya at a range of temperature 27 - 34°C. Each cat was kept in individual wire mesh cage 35 cm length, 28 cm width, and 30 cm height. Beneath each cage there was a dropping pan of plastic material which was cleaned every day [Figure 1]. They were fed once a day with friskies commercial cat food which they adapted to from the first day. A container of clean water was provided in each cage.

Figure 1.Domestic cat used in the experiment housed in a cagein the animal house.

These cats infected with *Spirometra*sparganawere the source of eggs usedfor validation of cat as a definitive host in this study. Eggs were detected using faecal smear, floatation and sedimentation methods from faeces of cats post infection with spargana. This was done from the 5th day post infection to the day eggs appeared for the first time in faeces of experimental cats. Later the sedimentation method was used to collect eggs for all the necessary studies.

Faecal smear

Using a wooden spatula, a small amount of faeces was placed on a drop of water on a slide, mixed well, and spread evenly over a small area. Then a cover slip was placed on top of the slide and examined under light microscope at 40x magnification.

Sedimentation method

Faeces collected from each cat was transferred into a separate beaker with tap water then mixed well with a glass rod until all lumps were broken. The mixture was sieved by using 4 sieves No.250, 150, 75 and 53 µm. Eggs were washed with tap water according to Vogel (1970). Contents of the beaker was left for 5 minutes so that the eggs can settle down. Water was poured out and new water was added into a beaker. This procedure was repeated several times until the water in the beaker was clean. The sediment was sucked with a Pasteur pipette and transferred into a petri dish where water was added and the contents examined under light microscope at 40x magnification. The eggs were transferred into bottles, and stored in a fridge at 4ºC. Some eggs were used fresh for other studies.

Recovery of Adult Worm from cats Experimentally Infected with spargana from Hamster Mice

After 575 days (18 months) two cats were sacrificed to recover adult worms. Experimental cats were transferred from the Animal House of the Institute of Biological Sciences to the Animal House of the Faculty of Medicine which has facilities for euthanasia and dissection. Cats were first prepared before dissection. Each cat was pre-anaesthetized with Ketamine 20 mg/kg IM + Xylazine 1.1 mg/kg IM, and then euthanized with Dolethal35 ml IV. When each cat was fully euthanaezed, was placed on a dissection table, a midline incision on the abdomen was made and opened in layers. Distal part of small intestine was identified, opened starting from this part to avoid damaging the neck and scolex of the worm. The adult worm was identified in the lumen of the small intestine. It was traced towards the duodenum until reached the site of attachment of the scolex. To locate the level of attachment, measurement was taken from this site to the pylorus. A circular cut was made around the site of attachment to make sure the scolex is not left behind at the site of attachment when removing the strobila from the lumen. Worms were placed in plastic containers with Normal Saline and transferred to the Institute of Biological Sciences for processing.

(d) Morphological Study of Eggs

The eggs collected were used for morphological studies using light microscope.

(i) Light microscope

Fresh eggs collected from faeces of cats were washed with tap water by changing water in the beaker several times until the water was clear. Eggs were transferred with Pasture pipette from beaker to Petri dish. Under dissecting microscope eggs were sucked with a small pipette and transferred on a slide. A drop of glycerine was added to a drop of water mixedwith eggs and slide cover was applied on top. The slide was left for about 15 minutes to let glycerineclean the egg shell. Then eggs were examined under light microscope.

(iii) Measurements of Eggs

A total of 200 fresh eggs collected from faeces of cats were measured length and width. About 1 ml of water mixed with eggs was sucked with a Pasteur pipette and placed in a petri dish. Under dissecting microscope eggs were sucked with a fine pipette transferred onto slides then slide covers wereapplied on top. The slides were examined under Leitz camera microscope where measurements of each egg were taken.

Collection and Preparation of Adult Worm for Morphological Studies

Adult worms recovered from small intestines of cats (definitive host) were first measured and then killed with hot 10% Formal Saline. Worms were placed between two glass plates and then kept in a plastic container with AFA (85% Alcohol + 10% Formalin + 5% Glycerol) overnight to be fixed.

(i) Measurement of Adult Worm

A total of 4 adult worms recovered from the small intestines of cats were placed in plastic containers with Normal Saline. They were left for some time in Normal Saline to relax. Then were picked with a forceps one after another from the containers, stretched on a graduated glass plate and the measurements of total length were taken and recorded as shown in (Table 1).

(ii) Morphological studies of Adult Worm

Scolex and part of the neck was whole mounted. Proglottids were taken from different parts of the strobila and whole mounted. The processed specimen were examined under light microscope.

Results

A total of 4 adult worms collected from experimentally infected cats, B, C and E were used for the morphological studies. The worms were long, segmented, with small head, long and thin neck. The scolex was small, spatulate and elongated.The worms were stained with Alum Carmine stain and examined under light microscope. Two lateral bothria in the scolex were visible. The proglottid showed centrally placed uterus with 6 coils. In the strobila the sexual organs were visible, namely the uterus and the testis, vitelleria and the 3 openings to the surface, namely genital opening, vaginal opening and the uterine opening. The genital pore was located on the upper ventral side, the vaginal pore, a short distance in front of the terminal uterine coil and the uterine pore on the ventral side of the terminal coil and some distance from genital pore. Testes was not united anteriorly in the midline with the vitellaria.

Proglottids that were expelled in the faeces on the 81, 87, 332 and 377 days post infection. were stained with Alum Carmine stain and it was seen to contain many *Spirometra* eggs but uterus was not visible.

Table 1.Measurements of adult worm length

Worm	Length (cm)
1	15.0
2	47.5
3	53.5
4	16.0
Mean	**33.0**
Range	**15.0-53.5**

Development of Adult Worm in definitive host (Cat)

Spargana collected from experimentally infected hamster mice (fed with spargana collected from naturally infected *Ranacancrivora)* were fed to three cats and the development observed. On necropsy of two cats, a total of 3 adult worms of *Spirometra* were recovered from the small intestines of the infected cats. This confirms our postulation that cats are the definitive host. The worms were patent and fully developed eggs present in the strobila. The pre-patency period of the cats varied as Cat A produced eggs in the faeces onthe 14, Cat B on the12and Cat C on the 60day.

Discussion

This study elucidates the role of the domestic cat as a definitive host and laboratory model for life cycle studies of *Spirometra*species. Early studies [7, 8]showed the presence of *Spirometra* infection in cats, bobcats, dogs and other carnivores.This study has established the domestic cat to be a suitable definitive host of *Spirometra*species. A single cat was observed to accommodate 1 to 2 worms, and the attachment of the scolex was usually along the middle third of small intestine, away from the pyloric valve. This conform with the findings of Mueller [8] who reported similar results with adult *Spirometramansonoides* in adult cats. The earlier studies show the presence of 3-4 worms which may be due to the fact that these cats were given a large dose of spargana but the location of worms are similar. The micro-environment of the adult parasite inside the definitive host which is the small intestine is important for its survival. McIntosh [7] has studied carnivores and reported to have found the worms in the small intestine of the cats, bobcats and dogs. Muller [8] found the head of the adult worm to be attached usually along the middle third of the small intestine in older cats and in kittens forward about 2cm posterior to the pyloric valve. He also reported that the entire strobila measured 25cm (10inches) or less in small hosts and that it may grow to 1.5m (5 feet) in adult dogs. He has also stated that a single cat may accommodate 3-4 worms, but as the number increases, competition and crowding effects may stunt the worms. In the present study three adult cats(named A, B, and C) which were orally infected with spargana were sacrificed on the 139 and 575 days post infection. One cat (name the cat A, B or C) was found to accommodate two adult worms and the other two cats each was found to accommodate a single adult worm. The site of attachment was far from the pyloric valve. In Cat B there was a single worm that was found 24 cm from the pylorus, in Cat C there were two worms found 26cm from the pylorus and in Cat (E ?)it(there)was a single worm found 60cm from the pylorus. This conformedwithMueller's study[8] who reported the attachment of adult *S. mansonoides* along the middle third of the small intestines in older cats. Also the measurements of the entire adult worms in mythisstudy were rangedbetween 15.0-53.5 cm (6.0-21.4 inches) and these measurements arewerewithin the range of measurements of adult *Spirometra* when grown in cats as reported by previous researchers.Morphology of adult worm of *Spirometra*has been studied [9, 10]. They reported that scolex has two bothria, neck long, uterus coiled with 5 to 7 coils, it occupies a narrow field along the median line. Uterine pore lies in the midline some little distance behind the anterior margin of the male genital pore. Vaginal pore is broad, just behind the male genital pore. Testes and vitellaria do not unite anteriorly in the midline. Mueller [8] reported that the uterus of *S. mansonoides* differs from *S. erinacei*, it is coiled with **C**-shaped outer loop of the uterus, and its anterior limb constricted in midline forming a

lateral expulsion chamber. In the present study, the morphology ofthescolex is spatulate with two lateral bothria, neck long, mature proglottid has 3 pores: genital pore located on the upper ventral side of the proglottid, vaginal pore located short distance in front of the terminal uterine coil, and uterine pore lies on the ventral side of the terminal coil some distance further from the genital pore. Uterus has 6 coils, centrally located in the proglottid. Testes were not united with vitellaria in the midline. The results of the present study corresponded with the results of the previous researchers for *S. erinacei.*

Conclusion

This study elucidated thatdomestic catsisarea definitive host of *Spirometra* species. An experimental study was carried out in the laboratory. Domestic cats were fed orally with spargana which developed to adult worms. The eggs were recovered, studied for its morphometrics and morphology. Adult worms were recovered from the small intestines of the domestic cats. The morphology of the adult worms was determined. All the findings were compatible with adult *Spirometra* species. The results concludes that domestic cats are definitive hosts of*Spirometra* species.(delete this section)

Ethical issue

There is no ethical issue in this study because no human material was used.

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