Survey of *Spirometra erinaceieuropaei* in frogs in Taiwan and its experimental infection in cats

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Abstract

Eighteen of 56 (32.1%) wild *Rana limnocharis* from central and south Taiwan were found to contain plerocercoids of *Spirometra erinaceieuropaei*. This is the first report of *S. erinaceieuropaei* infections in frogs in Taiwan, with the plerocercoids being recovered from the thigh and back muscles or under the skin. Other species of frogs examined, including nine wild *R. latouchii*, one wild *Buergeria robustus* and 110 cultured *R. rugulosa* were free of infection. The plerocercoids were orally inoculated into four cats; three of which were each given a single plerocercoid and one a dose of three plerocercoids. Daily faecal examination showed that two cats started shedding eggs of *S. erinaceieuropaei* on day 8 postinfection (PI) and the other two on day 10 PI. The highest eggs per gram and eggs per day for a single worm was found to be 428,000 and 14,416,000 respectively. Only the cat inoculated with three plerocercoids shed proglottids in its faeces during the 2 month observation period.

Introduction

Sparganosis is caused by plerocercoids of the diphyllobothrid tapeworm, *Spirometra erinaceieuropaei* (Rudolphi, 1819) Mueller, 1937 (synonym *Spirometra erinacei*) (Wardle & McLeod, 1952). It is a form of visceral larva migrans. Infections in humans occur mainly through the ingestion of undercooked or uncooked meat of second intermediate/paratenic hosts such as frogs, snakes and chicken (Lee *et al*., 1975; Beaver *et al*. 1984). Drinking unboiled water contaminated with infected copepods which serve as the first intermediate host is also a possible route of infection (Lee *et al*., 1975). Ocular sparganosis in humans due to the practice of applying raw frog flesh as a poultice to irritated eyes has been reported in Thailand (Kittipongghansa *et al*., 1988). The similar practice of applying a poultice of raw snake flesh to the site of a painful abdominal hernia has resulted in abdominal sparganosis being reported in Ecuador (Kron *et al*., 1991). In these cases, plerocercoids were able to enter the human body via the percutaneous route.

In humans, the larvae migrate to various visceral organs (Tanaka *et al*., 1997) and also subcutaneous tissues causing swellings over the entire body (Garin *et al*., 1997; Moreira *et al*. 1997), fevers and occasionally fatality when the brain is involved (Chang *et al*., 1992). In Taiwan, following the first report of human sparganosis by Suemori (1922), more than a dozen human cases, which include osseous, cerebral and subcutaneous sparganosis, have been documented (Wang & Cross, 1974; Lin *et al*., 1978; Liao *et al*., 1984; Lo *et al*., 1987; Chung *et al*., 1990; Chen *et al*., 1992; Tsai *et al*., 1993; Tsou & Huang, 1993). These cases could be related to the fact that frogs and snakes are widely eaten by people in Taiwan. These frogs and snakes are suspected to harbour the plerocercoids of *S. erinaceieuropaei* because comparatively high infections by this cestode were reported in cats in Taiwan (Tsai *et al*.,

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1996). Thus, from a public health viewpoint, a survey of the occurrence of this parasite in amphibians and reptiles in Taiwan is required.

In this paper, we present the results of a survey of plerocercoids of *S. erinaceieuropaei* in frogs from Taiwan and preliminary experimental infections in cats.

Materials and methods

Collection and examination of frogs

Wild frogs were caught from a field site in the Taichung area, central Taiwan from October 1997 to November 1998. Farm-raised frogs were purchased from markets in central and southern Taiwan. The frogs were killed using ethyl-ether anesthesia, weighed, skinned and their skeletal muscles and skin carefully examined for plerocercoids. A total of 176 frogs comprising 56 *Rana limnocharis*, nine *R. latouchii*, 110 *R. rugulosa* and one *Buergeria robustus* were examined. All the *R. rugulosa* were aquacultured and purchased from the markets.

Experimental infections in cats

Plerocercoids collected from frogs were kept alive in RPMI 1640 culture medium supplemented with 2% foetal calf serum and stored at 4°C until used for infection in cats.

Four one-year-old cats were treated with praziquantel at a dose of 5 mg kg^{-1} body weight and their faeces checked for at least 2 weeks to confirm the absence of tapeworms in the intestine. Three plerocercoids were orally inoculated into one cat, and the remaining three cats were each given a single plerocercoid. The faeces of the inoculated cats were examined almost every day for two months. Eggs per gram of faeces (EPG) were determined for all the faecal samples collected, whereas the eggs per day (EPD) for each cat was calculated by multiplying the total amount of faeces deposited per day with the EPG. Thus, for cats infected with only a single worm, the EPD represents the amount of eggs shed by a single worm for that day.

Morphological examination of proglottids

Proglottids shed with the faeces were collected and fixed in acetic acid–alcohol after relaxation in an ice water bath. The proglottids were then flattened between two slide glasses, stained with alum–carmine, destained in 1% hydrochloric acid in 70% alcohol, dehydrated through an alcohol series, cleared in xylene and mounted in Entellan® (Merck Co.) to confirm the species identification.

Results

Plerocercoids of *S. erinaceieuropaei* were found in 18 of 56 (32.1%) of wild caught *R. limnocharis* but no other frog species was found to be infected. The majority of plerocercoids were found in the femoral muscle or subcutaneous connective tissues of the hind limb. A few plerocercoids were also seen in the dorsal muscles or under the skin (table 1).

The cat inoculated with three plerocercoids and the other with a single plerocercoid began shedding eggs on day 8 postinfection (PI). The remaining two cats also started to shed eggs on day 10 PI. Thus, the prepatent period of *S. erinaceieuropaei* in cats in Taiwan is 8 to 10 days. Irregular peaks were observed in the daily EPG (fig. 1). One cat (cat C) which was fed with a single plerocercoid continuously deposited faeces without worm eggs for 4 consecutive days starting from day 30 PI. The kinetics of eggs shed per day in the faeces of the four cats are shown fig. 2. The highest EPG and EPD for a single worm was observed to be 428,000 and 14,416,000, respectively.

Table 1. Predilection sites of *Spirometra erinaceieuropaei* plerocercoids in *Rana limnocharis**.

Site	No. of plerocercoids (% occurrence)
Left fore leg	2 (2.7%)
Right fore leg	2 (2.7%)
Left hind leg	22 (29.3%)
Right hind leg	43 (57.3%)
Dorsal muscle	6 (8%)

*n = 18.

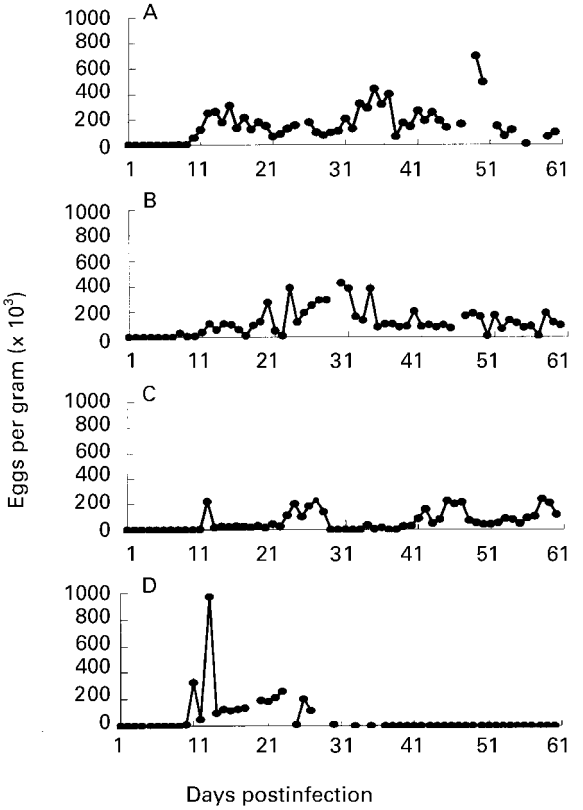

Fig. 1. Kinetics of daily EPG (eggs per gram) in the faeces of cats experimentally fed with three plerocercoids (A) or one plerocercoid (B, C and D) of *Spirometra erinaceieuropaei*. Lack of data on the graph indicates no faeces was collected on that day.

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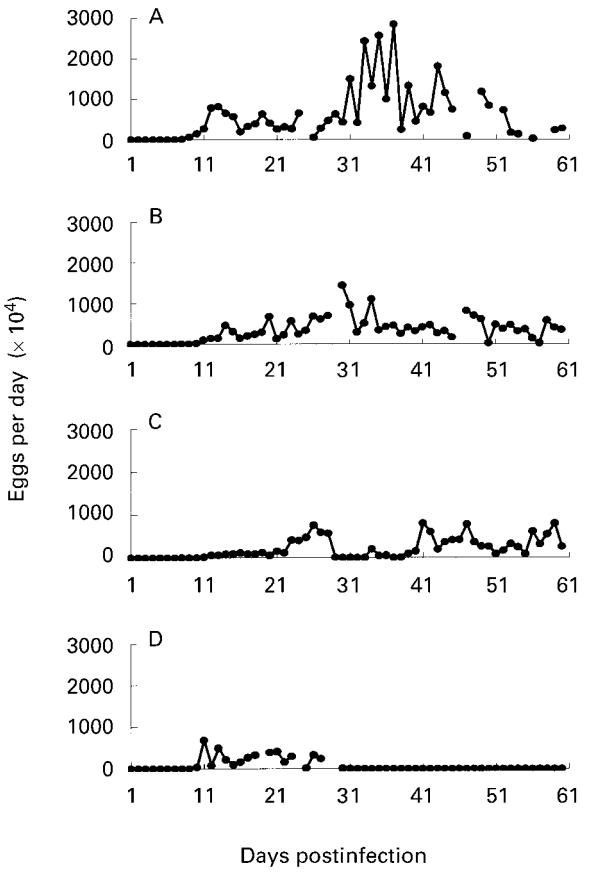

Fig. 2. Kinetics of EPD (eggs per day) in the faeces of cats experimentally fed with three plerocercoids (A) or one plerocercoid (B, C and D) of *Spirometra erinaceieuropaei*. Lack of data on the graph indicates no faeces was collected on that day.

Only the cat inoculated with three plerocercoids deposited proglottids in its faeces on days 24, 26, 28, 30, 41 and 55 PI. In the cat (cat D) fed a single plerocercoid, no eggs were detected in the faeces after day 33 PI, suggesting that the worm had been expelled or degenerated.

Morphological examinations of expelled proglottids showed that the uterus was coiled 2–3 times, thus confirming that the species was *S. erinaceieuropaei*.

Discussion

This is the first report of *S. erinaceieuropaei* plerocercoid infections in Taiwan. Our observation that *R. limnocharis* is susceptible to *S. erinaceieuropaei* concurred with the findings of Mastura *et al*. (1996) in Malaysia. This may be due to the ecology of this frog species which prefers to dwell in lowland areas with stagnant water bodies which are suitable for the growth and propagation of cyclopoid copepods, which are known to serve as first intermediate hosts of *S. erinaceieuropaei*. The frog presumably becomes infected by ingesting infected copepods during its tadpole stage. Experimental transmission of *S. erinaceieuropaei* from infected *Mesoclyclops leuckarti* and *Eucyclops serrulatus* to tadpoles of *R. nigromaculata* was previously demonstrated by Lee *et al*. (1990).

In their study on the prevalence of plerocercoids in *R. nigromaculata* in South Korea, Kim & Shin (1975) observed that more than 82% of the plerocercoids were distributed in the femoral muscle and connective tissues of the hind legs. In the present study, up to 87% of the plerocercoids were found in the hind legs of *R. limnocharis*, suggesting that the hind legs are the preferred sites of predilection of the plerocercoids in frogs. The thighs provide soft tissues for the plerocercoids to migrate to following the metamorphosis of tadpoles to frogs.

From a public health viewpoint, it is noteworthy that no plerocercoids were detected in frogs on sale for human consumption. Presumably these aquacultured frogs, even during their tadpole stage, were kept in concrete ponds and their chances of exposure to *S. erinaceieuropaei* infected copepods were limited. However, further monitoring of amphibians maintained under aquaculture condition is warranted.

The prepatent period of *S. erinaceieuropaei* has been reported to be 15 days in cats (Lee *et al*., 1990). Although the present study showed a shorter prepatent period of *S. erinaceieuropaei* in cat (8–10 days), such a difference may be due to biogeographical factors involved in the transmission of this cestode species.

The fact that proglottids were expelled from the cat inoculated with multiple plerocercoids, but not from those inoculated with a single plerocercoid, suggests that some interaction occurs between the worms themselves, such as competition for nutrients, which in turn limits the total worm mass within intestine. If such a phenomenon can be equivocally proved, then it augurs well for both the parasite and the host in that symptoms such as ileus or obstruction of the intestine could be reduced.

The daily EPG and EPD of cats inoculated with either a single or three plerocercoids showed irregular peaks. As eggs were not detected in the faeces of the infected cat on some days, this implies that faecal examination for the diagnosis of cestode infections in mammals should be carried out on several consecutive days before final conclusions on diagnosis are drawn.

Thousands of eggs were shed in cat faeces daily as reflected by the high EPD, suggesting that a single worm will be sufficient to contaminate the environment. The public health implication of this observation is that human sparganosis had been suggested to occur through drinking water which contained eggs of *S. erinaceieuropaei* which subsequently were able to hatch, infect copepods and then develop into procercoids (Chung *et al*., 1990).

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