A SURVEY OF EPIDEMIOLOGY OF *GNATHOSTOMA HISPIDUM* AND EXPERIMENTAL STUDIES OF ITS LARVAE IN ANIMALS

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Abstract. The present study is concerned with a survey of the epidemiology and infectivity of *Gnathostoma hispidum* larvae in animals. A total or 3,478 pigs were examined in Hongze-Lake, Jiangsu; Po-Lake, Anhui; and Jiujiang, in Jiangxi Province. The infection rate of *G. hispidum* in pigs was 14.9%. The prevalence in pigs in the lake districts (26.4%) was considerably higher than in the mountainous districts (5.1%). The results of field surveys and experimental studies revealed that in China, 38 animal species served as the first and the second intermediate hosts and paratenic hosts of *G. hispidum.* Among them, 23 species (6 cyclops, 13 fishes, 2 frogs and one each of snake and bird) were similar to the hosts of *G. spinigerum.* Carnivorous fishes, frogs and rodents are an important infection source for gnathostomiasis. Experiments demonstrated that *Macaca mulatta* was successfully infected with the early 3rd-stage larvae from cyclops or the advanced 3rd-stage larvae from fish. The domestic cat and white rat were experimentally infected by skin penetration by advanced 3rd-stage larvae of *G. hispidum* obtained from fish; the results of the test were all positive. The survey of epidemiology and biology of *G. hispidum* and *G. spinigerum* were quite similar, so the authors consider that humans may be infected by *G. hispidum.*

INTRODUCTION

Gnathostoma hispidum is widely distributed in the world. In China, the worm has been found in Xinjiang, Henan, Zhejiang, Jiangsu, Anhui, Hubei, Yunnan, Jiangxi, Hunan, Guangdong, Guangxi, Fujian and Taiwan provinces. The infection rates in pigs were 0.6% (Chen, 1936) in Guangzhou, Guangdong; 5.1% (Wang *et al,* 1976) in Fuzhou, Fujian; 7.9% (Huang *et al,* 1986) in Jiangxi; 55.5% (Wang *et al,* 1986) in Changde, Hunan. The parasite has not yet been thoroughly studied epidemiologically in China. From 1980 to 1984, a survey of epidemiology of *G. spinigerum* was carried out in Hongze district, Jiangsu Province. In our examinations, *G. hispidum* larvae have been found in many species of fishes. Akahane (1982) reported that *Misgurnus anguillicaudatus* imported from Heijing and Nanjing, China was infected with the larvae. Morishita (1924) and Chen (1949) each reported a case of human gnathostomiasis. At that time, these two cases were considered to be caused by *G. hispidum* but Miyazaki (1960) was very skeptical about the identification of the parasite by Morishita and Chen. Thus it has not yet been made clear whether man can be infected by the parasite. Recently Morita *et al* (1984) reported 8 cases of human gnathostomiasis which were caused by eating raw loaches containing *Gnathostoma* larvae. It is considered that some of the recent gnathostomiasis occurring in Japan may be caused by this nematode, but no *G. hispidum* has been found yet in human patients (Ando *et al,* 1988). In order to understand the endemic status in animals in China, we carried out this study in 1988.

MATERIALS AND METHODS

The various animals examined were collected in endemic areas, such as Hongze-Lake district and Suson County, Anhui. All the animals tested were from the non-endemic region and they were examined to be negative for *G. hispidum*. The final host was examined for *G. hispidum* by means of direct autopsy. The viscera of the fishes, frogs, snakes and birds were directly torn under a dissecting microscope and examined for natural infections with *Gnathostoma* larvae. The muscles, pounded to pieces, with added artificial digestive juice, were placed at a constant temperature of 36-37°C for 24 hours for digestion. Then they were washed in tap water and the precipitates were examined for *G. hispidum*. All larvae were discriminated by microscopic examination. The experimental animals (monkeys, cats and rodents) were anesthetized by Injectio Ketamini Hydrachloridi before being tested. The cats and rats were restrained on a board, which permitted examination of the study areas with a dissecting microscope or a magnifying glass. The intact skin preparation involved shaved skin : the scratched skin preparation involved scratching the shaved area superficially to the depth of the subcutaneous tissue with a dissecting needle. The experimental animals were fed various numbers of fully developed *G. hispidum* larvae in cyclops and advanced 3rd-stage larvae from fish or other infected vertebrates.

RESULTS

Epidemiological examinations

Examination of definitive host: To determine the incidence of *G. hispidum* in the definitive host, the examinations of 3,478 pigs stomachs were carried out at slaughter houses. The results are shown in Table 1.

Surveys of the second intermediate hosts and paratenic hosts : During 1980-1987, a survey of epidemiology was conducted in Hongze-Lake, Jiangsu Province and Po-Lake, Susong County, Anhui Province. A total of 1,325 fish belonging to 14 families, 31 genera and 34 species were examined and 14 species were found to be naturally infected with the advanced 3rd-stage larvae of *G. hispidum* (Table 2). The results showed that the infection rate and intensity of the second intermediate hosts of both *G. hispidum* and *G. spinigerm* (Lin and Chen, 1986) were highest in the carnivorous fishes. The infection rate of *G. hispidum* larvae reached as high as 66.2% in *Monpterus* and the mean number of larvae per fish was 37.6. It is clear that in the endemic areas, these fish may play a significant role in spreading *G. hispidum* disease.

The morphological characters (Figs 1-3) of *G. hispidum* advanced 3rd-stage larva from naturally infected fish were as follows : The larvae (n = 20) were 2.258 ± 0.703 × 0.284 ± 0.031 mm in size. The larva's head bulb had four rows of hooklets, the numbers of which were 36 ± 3 on the first, 39 ± 6 on the second, 40 ± 6 on the third and 42 ± 6 on the last row. The entire body of the larva was covered with many transverse rows of single pointed spines directed posteriorly the number of which was 210 ± 35. One pair of cervical papillae was situated between the 10th-14th trans-

Table 1

Examination of *G. hispidum* in pigs.

Year of examination	Locality	Number examined	% Positive	Intensity of infection (average)
1985-1986	Hongze-Lake Jiangsu	399	34.9	1-15 (4.2)
1987-1988	Po-Lake Anhui	1,213	23.6	1-18
1986-1987	*Jiujiang Jiangxi	1,867	5.1	1-34 (3.7)

* Mountainous district.

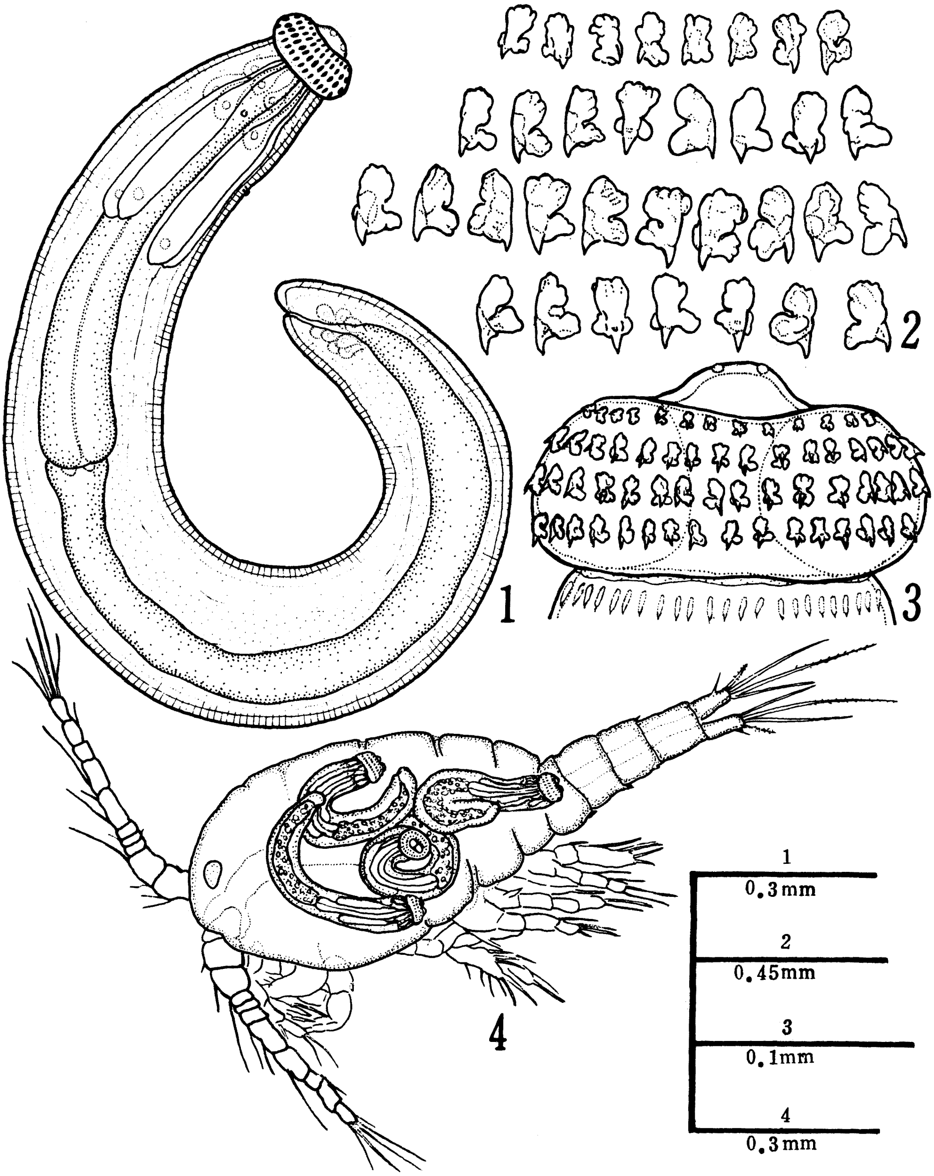

Fig 1-4—Morphology of *Gnathostoma hispidum* larva. 1. Advanced 3rd-stage larva from fish. 2. Magnification of 4 rows of hooklets on the head bulb. 3. Magnification of head bulb of advanced 3rd-stage larva, showing 4 rows of hooklets. 4. Early 3rd-stage larva in the *Apocyclops royi*.

verse rows and the excretory pore situated between 19th-24th transverse rows.

In the same regions, about 11 species of paratenic hosts were examined and 6 species found to be naturally infected with the larvae (Table 3). The above surveys indicated that rodents and snakes are the main sources of infection for *G. hispidum*.

Artificial tests of animals

Test of the first intermediate host: Fertilized eggs of *G. hispidum* were obtained from stools of the

Table 2

Natural infection of 14 species of freshwater fishes with *G. hispidum* advanced 3rd-stage larvae.

Species	No. examined	% Positive	Intensity
Cyprinus caroio	36	5.6	1
Carassius auratus	49	6.1	1-3
**Opsariichthys uncirostric bidens*	89	11.2	1-15
**Hemibarbus maculatus*	13	15.4	1-2
**Culter erythropterus*	15	40.0	1-9
**Erythroculter iliskaeformis*	10	10.0	2
**Parasilurus asotus*	20	40.0	1-8
Misgaurnus anguillicaudatus	153	7.8	1-7
**Psudobagrus fulvidraco*	69	26.1	1-10
**Anguilla japonica*	35	11.4	1-3
**Ophiocephalus argus*	47	31.9	1-48
**Monopterus albus*	281	66.2	1-732
**Siniperca chuatis*	43	25.6	3-13
**Odontobutis obscurus*	51	23.5	1-21

* Carnivorous fish.

Table 3

Natural infection of 6 paratenic hosts species with *G. hispidum* advanced 3rd-stage larvae.

Species	No. examined	% Positive	Intensity
Rana limnocharis	490	3.1	1-3
R. nigromaculata	158	12.7	1-4
R. tigrina rugulosa	24	50.0	1-3
Elaphe rufodorsata	8	100.0	5-107
Gallus gallus domesticus	20	20.0	1-3
Rattus norvegicus	10	30.0	7-35

infected pig. Fresh eggs were kept in tap water at room temperature of 28-30°C. Sheathed larvae hatched after 7-8 days. The various cyclops were examined 12-14 days after exposure. The results proved that there are 10 species of cyclops acting as the first intermediate host of *G. hispidum*, namely *Cyclops sternuus*, *C. vicinus*, *Mesocyclops leukart*, *Eucyclops serratus*, *Thermocyclops taihokuensis*, *Apocyclops royi*, *Acanthocyclops viridis*, *Thermocyclops oithenoides*, *Thermocyclops hyalinus* and *Macrocyclops albidus*. *M. leukart* and *A. royi* (Fig 4) proved to be especially highly susceptible with an infection rate of 100% and they serve as an important intermediate host of *G. hispidum*.

Infection test of primates: To understand the infectivity of the early 3rd-stage larvae of *G. hispidum* in cyclops and the advanced 3rd-stage larvae obtained from fish for primates and to inquire into the problem of human infection, 3 *Macaca mulatta* were fed various numbers of the larvae from different hosts. The results are shown in Table 4. This result is generally in agreement with the experimental conclusion made by Daengsvang (1971) in primates with *G. spinigerum* larvae and it revealed that susceptibility to both *G. hispidum* and *G. spinigerum* larvae are the same in the primates.

Experiment of skin penetration: As yet there have been no studies reported on whether vertebrates can be infected by the mode of skin penetration with *G. hispidum* larvae. In order to determine whether animals and man are infected by *G. hispidum* larvae through the route of skin penetration, 3 domestic cats and 3 white rats were experimentally infected by skin penetration, using both the early 3rd-stage larvae of *G. hispidum* in cyclops and the advanced 3rd-stage larvae from infected fish hosts. The results showed that both intact and scratched skin of experimental animals of the two kinds could be infected, but only the advanced 3rd-stage larvae obtained from fish possessed the power of infectivity or penetration. The penetration rate of larvae (No. of larvae observed penetrating into the skin/No. of larvae exposed on the skin) in both the intact and scratched skin was 100% for the white rats; in the intact skin was 16.7% (2/12) and in scratched skin wàs 100% for the cat. The recovery rate of larvae (No. larvae found in organs/No. of larvae penetrating into the skin) in white rats autopsied and examined 14 days after infection was 50% (7/14), with 6 larvae parasitized in muscles of the fore limbs, 1 in viscera; in the cats killed and examined 21 days after infection, it was 10% (2/20), the larvae being parasitized in muscles of hind limbs. Our experiments suggested that humans can possibly become infected as the experimental animals did.

Table 4

The results of feeding *G. hispidum* larvae to *Macaca mulatta.*

No. of animals (days until autopsy after feeding)	1 (57)	2 (224)	3 (57)
No. of larvae fed (source of larvae)	250 (fish)	600 (fish)	821 (cyclops)
Number and location of larvae found			
spleen	0	1	0
pancreas	0	0	1
liver	1	0	3
diaphragm muscle	0	2	1
abdominal muscle	3	2	0
thoracic muscle	10	19	0
cervical muscle	2	12	0
lumber muscle	27	43	1
muscle of left fore limb	0	48	2
muscle of right fore limb	1	40	0
muscle of hind limb	1	7	1
Total number of larvae found	46	191	9

Infective experiments of other mammals: Daengsvang (1973) tested 4 kinds of mammals and found 2 rodent species acting as the paratenic hosts for *G. hispidum.* The recovery rate of larvae was 27% at autopsy after 3-348 days. In our tests, 5 mammalian species, *Mus bactrianus albula, Ruttus norvegicus, Caviva porcellus, Meriones unguiculatus* and *Suncus murinus,* were orally infected with the advanced 3rd-stage larvae obtained from fish and infected ducks. The experimental animals were sacrificed and examined 5-58 days after infection. They were all found to be infected and the larvae had a slight increase in size but no further morphological changes. The recovery rates of the larvae (No. of larvae found in organs/No. of larvae fed) in order were 41.7% (10/24), 12.5% (10/80), 22.2% (4/18), 12% (6/50) and 64.9% (24/37), respectively. Test results showed that both *Suncus murinus* and rodents seemed to be suitable paratenic hosts for the parasite, indicating that these may be an important infection source for gnathostomiasis. In prevention, attention must be paid to this problem.

Infective tests of birds: Birds acting as paratenic hosts for *G. hispidum* have been unknown except that Daija (1969) reported 3 wild birds species and *Gallus gallus domesticus,* these four species to be naturally infected with the larvae. To determine infectivity of *G. hispidum* larvae for birds, 4 bird species were tested using the advanced 3rd-stage larvae from fish and infected monkeys. The results proved that 4 animal species ie *Gallus gallus domesticus, Anas platyrhyncus domesticus, Streptopelia chinensis* and *Acridotheres cristaellus* were successfully infected. The recovery rates of larvae were 23.3% (14/60), 18.8% (13/69), 6.7% (2/30) and 6.7% (2/30) respectively at autopsy 3-11 days after infection. The experiments showed that both domestic fowls and wild fowls could serve as paratenic hosts for the parasite. Daengsvang (1973) reported a case of gnathostomiasis which was caused by eating partially cooked chicken containing *G. hispidum* larvae.

DISCUSSION

As yet, the views of the problem regarding human infection by *G. hispidum* and inconsistent among various investigators (Morishita, 1924; Chen, 1949; Miyazaki, 1960; Daengsvang, 1973; Morita, 1984; Ando *et al,* 1988). Our results support Morita's report and consider that humans may be infected by eating uncooked fish or paratenic hosts containing *G. hispidum* larvae or by drinking water carrying the larvae in cyclops. The reasons are as follows : 1. According to the studies of Daengsvang (1976), Wang *et al* (1986) and the authors (1987; 1990) the life cycle of *G. hispidum* was shown to be similar to that of *G. spinigerum* reported by Prommas and Daengsvang (1933; 1936; 1937). Their first and second intermediate hosts and paratenic hosts are almost the same. 2. A great many kinds of animals act as vectors of transmission for the two parasites, which are transmitted mainly by oral infection. From the data above, the authors consider that the biological characters of these two *Gnathostoma* species are very similar. 3. It has been shown by the authors that *Macaca mulatta* is susceptible to the early 3rd-stage larvae in cyclops and advanced 3rd-stage larvae from fish. Analysis of the status of the prevalence of *G. hispidum* in pigs revealed that in China there are two types of endemic regions, namely the lake districts, such as Po-Lake, Anhui, Hongze-Lake, Jiangsu and Changde, Hunan (near the Dongthing-Lake) and the mountainous districts, such as Guangzhou, Guangdong and Fuzhou, Fujian. In the lake districts, because these places have abundant resources of water and fish and provide suitable conditions for this parasite to complete its life cycle, and because pigs in these areas are mostly put out to pasture, which increases their exposure to infection sources of *G. hispidum,* the prevalence of *G. hispidum* in pigs is high in both infection rate and concentration. In the mountainous districts where there are less ideal conditions for the worm to complete its history, pigs have much less exposure to infection, so although the pigs roam freely, the infection rate of the parasite is low and scattered.

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