DEVELOPMENT OF ADULT *GNATHOSTOMA SPINIGERUM* IN THE DEFINITIVE HOST (CAT AND DOG) BY SKIN PENETRATION OF THE ADVANCED THIRD-STAGE LARVAE

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INTRODUCTION

The infection of cats by orally administered advanced third-stage *Gnathostoma spinigerum* larvae was first successfully demonstrated by Prommas and Daengsvang (1937). Early attempts by these workers to detect active skin penetration by newly hatched larvae were unsuccessful (1933). However, Daengsvang (1968) has shown prenatal transmission of the parasite in mice, and recently, Daengsvang, Sermswatsri, Youngyi and Guname (being prepared for publication in the *Ann. Trop. Med. Parasit.*) described penetration of the skin of mice, rats and cats by advanced third-stage larvae. However, fully developed larvae, obtained from experimentally infected cyclops, were unable to penetrate the skin of mice.

There have been no studies reported dealing with the development of the advanced third-stage larvae from the time of penetration until a patent infection develops. The present report is concerned with additional observations on skin penetration by advanced third-stage larvae in eight adult domestic cats and six dogs with resulting development of the adult worm in the stomachs and the passing of eggs in the stools.

MATERIALS AND METHODS

A total of 14 adult domestic cats and dogs (8 cats and 6 dogs) were used; none of these animals exhibited evidence of infection by gnathostomes prior to experimental infection. In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council.

The experimental animals were immobilized by general anethesia with ether or intravenous combuthal. The skin was prepared for the study by shaving a 8-10 cm square on the back of each of two cats and on the abdominal wall of the other cats and all dogs.

The *G. spinigerum* advanced third-stage larvae used were obtained from naturally infected snake-headed fish (*Ophicephalus striatus*) and experimentally infected white mice (*Mus musculus*). The larvae were placed on the skin and kept continuously moist by the frequent application of a few drops of water or by covering with water-soaked lens paper. The larvae were observed by the use of a magnifying glass until penetration was completed or for two hours in those cases in which complete penetration was not accomplished. The animals were then kept under close supervision and food, drink and cleanliness were controlled carefully. Weekly stool examinations by the formalin-ether

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concentration method, were performed on each experimental animal. At necropsy, a macroscopic search for larvae in the muscles and viscera was conducted using an electrical illumination apparatus. The identification of the worms found was always confirmed by microscopic examination.

RESULTS

Eight experimental cats showed the following: two cats were successfully skin penetrated on the back by 43 (83.0%) and 41 (100.0%) advanced third-stage larvae of *G. spinigerum* and began passing eggs in their stools about two months later, which was much earlier than the others. Necropsies four months after infection showed infectivity rates of 51.0% and 29.0% as measured by the presence of mature adult *G. spinigerum* in a small gastric tumor in each cat and many advanced third-stage larvae in the liver and diaphragm of both cats. In addition, larvae were found in the abdominal flesh of one cat and the leg muscles of the other. Two cats, the skins of which were penetrated by 42 (93.0%) and 85 (100.0%) larvae respectively, first became positive for eggs 6 and $7\frac{1}{2}$ months later. These animals are being kept for further study.

The skin of the other 4 experimental cats were successfully penetrated by the following numbers and percentages of larvae: 53 (62.0%), 46 (90.0%), 18 (100.0%) and 45 (75.0%). However, weekly stool examinations on these animals were negative for *G. spinigerum* up until the time of sacrifice (3 weeks to $6\frac{1}{2}$ months following exposure). The autopsies of these four infected cats gave the following results: two cats sacrificed 5 and $6\frac{1}{2}$ months after exposure to the larvae, showed infectivity rates of 51.0% and 56.0%; immature male and female worms were present in one gastric tumor, the abdominal flesh and the diaphragm of one cat and in the diaphragm and the flesh of the anterior chest wall of the other. There were also many advanced third-stage larvae found in various organs of the two animals. The other two cats, each sacrificed three weeks after the skin exposure, presented infectivity rates of 84.0% and 94.0% respectively; no adult worms were found but 38 and 17 *G. spinigerum* advanced third-stage larvae were present, mostly located in the livers, with a few in the diaphragm, and flesh and subcutaneous tissue on the anterior abdominal wall of one cat and in the peritoneal fat near the stomach of the other.

These observations on cats show clearly that many mature adult *G. spinigerum* can be found causing tumors of the stomach wall with a prepatent period ranging from 2 to $7\frac{1}{2}$ months after skin penetration by the larvae. However, many immature adult worms found in the diaphragm and in the anterior chest abdominal walls at 5 to $6\frac{1}{2}$ months following the successful penetration of 46 and 53 larvae through the skin of two cats. In about 3 weeks the advanced third-stage larvae had grown most slightly, being found in the liver but also in other tissue to some extent.

Six experimental adult domestic dogs were successfully skin penetrated by 64 to 192 *G. spinigerum* advanced third-stage larvae with a success rate of penetration of 39.0% to 100%. Weekly stool examinations first revealed *G. spinigerum* eggs in the stools of two dogs 3 and $3\frac{3}{4}$ months after exposure and at about 8 to $8\frac{1}{4}$ months in the four others. These results leave no doubt that mature adult *G. spinigerum* can develop in the gastrointestinal tract of the dog with resulting patent infection developing in 3 to $8\frac{1}{4}$ months after exposure (Table 1).

Table 1
Experimental skin penetrations on domestic cats and dogs by advanced third-stage larvae *of Gnathostoma spinigerum*.

No. and type of animal	No. times exposed	No. and % of larvae which successfully penetrated	Length of the prepatent period (days) range	Infectivity rate (%)	Days after patent infection developed
2 cats	2 & 6	41 (100.0%), 43 (83.0%)	59—71	29 & 51	63 & 60
		AUTOPSY RESULTS			
		One cat shows 6 mature male and female *G. spinigerum* in one gastric tumor (2.0 × 2.5 cm), 1 mature female in greater omentum and 5 advanced third-stage larvae of the worm in the liver, diaphragm and leg muscles. The other had 14 mature male & female *G. spinigerum* in one gastric tumor of 2.0 × 3.0 cm in size and 8 advanced third-stage larvae of the worm in the liver, diaphragm and flesh of the anterior abdominal wall.			
		REMARKS			
		Both infected through the skin of the back.			
2 cats	1 & 2	85 (100.0%), 42 (93.0%)	175-227	—	—
		RESULTS			
		Animals under observation.			
		REMARKS			
		In one cat all 85 (100.0%) larvae completed skin penetration in one hour. These 2 cats are being kept for further study.			
2 cats	1 & 3	46 (90.0%), 53 (62.0%)	Both negative for *G. spinigerun* eggs at sacrifice (154-195 days)	56 & 51	—
		AUTOPSY RESULTS			
		One cat exposed once to 46 larvae had 17 immature male and female *G. spinigerum* in the diaphragm and anterior chest wall flesh and 9 advanced third-stage larvae in the flesh of the anterior chest wall, abdominal wall, back and stomach. The other showed 12 immature adult male and female *G. spinigerum* in one gastric tumor (2.0 × 2.0 cm), and in the flesh of the anterior abdominal wall and diaphragm. There were also 15 advanced third-stage larvae of the worm in the peritoneal tissue near the stomach.			

Table 1 (Continued)

No. and type of animal	No. times exposed	No. and % of larvae which successfully penetrated	Length of the prepatent period (days) range	Infectivity rate (%)	Days after patent infection developed
2 cats	1 each	18 (100.0%), 45 (75.0%)	Both negative for *G. spinigerum* eggs at sacrifice (21 days after the exposure)	94 & 84	—
		AUTOPSY RESULTS			
		One cat had 17 *G. spinigerum* advanced third-stage larvae, mostly found in the liver; a few were found in the diaphragm, flesh of anterior abdominal wall, and the skin. The other cat had 38 advanced third-stage larvae of the worm most of which were found also in the liver with a few in the anterior abdominal flesh.			
6 dogs	1 to 3	64 to 192 (39.0% to 100.0%)	84 to 247	63 (one dog)	31
		AUTOPSY RESULTS			
		One dog exposed once to 65 larvae (79%) became positive for *G. spinigerum* eggs 231 days later. One month afterwards 37 mature adult male and female, and 2 immature female *G. spinigerum* were found in one gastric tumor of 3.0 cm in diameter, 1 immature female each in the peritoneal tissue near the stomach and in the upper part of the thorax.			
		REMARKS			
		5 infected dogs kept for further study.			

DISCUSSION AND SUMMARY

These studies clearly show that advanced third-stage larvae of *G. spinigerum* can penetrate through the intact skin of cats and dogs. All eight experimental cats were successfully infected with rates of 62.0% to 100.0%; all six experimental dogs were also infected, with penetration rates of 39.0% to 100.0%. In one cat, 85 larvae (100.0%) completed the process in only one hour. In general, the present observations on the total time and true penetration time required by the larvae to penetrate through the intact skin of cats and dogs showed no significant differences from those described in the previously reported on

cats by the same workers (being prepared for publication in the *Ann. Trop. Med. Parasit.*); about 15.0% of the larvae exhibited a total time (the time required from initial contact with the skin to complete penetration of the larvae) of about 30 minutes and the true penetration time for the whole group (the time recorded from the moment the head of each larva began to penetrate until the disappearance of the posterior end through the skin) ranged from about 5 to 80 minutes. After completing skin penetration, the larvae were next found in the subcutaneous tissue and the nearby muscles; later they were discovered mostly in the liver, and subsequently in the diaphragm, abdominal tissue and the chest wall with no difference between cats exposed on the abdominal skin and on the back except that one larva was located in the right hind leg muscles of one cat exposed on the skin of the back.

Of the eight experimental cats, two infected by the larvae through the back skin showed the first egg positive stools at about two months, while the other two cats became positive for eggs in about 6 and 7 $\frac{1}{2}$ months. The remaining 4 cats were found to be infected with either immature worms or larval stages which were found in many organs on autopsy 3 weeks to 6 $\frac{1}{2}$ months following exposure. Thus maturity of the worms in the stomach walls of cats is developed with a still unexplainable variation of the time interval between skin penetration by the larvae and development of adult worms capable of shedding eggs in the stool. In different cats this interval ranged from about 2 to 7 $\frac{1}{2}$ months.

The infectivity rate of larvae successfully penetrating the intact skin, as shown by established infection by adults and or larvae in experimental cats sacrificed on various periods following exposure, varied from 29.0% to 94.0%.

All experimental dogs skin penetrated by the larvae showed on weekly examinations the first *G. spinigerum* egg positive stools also at greatly variable time intervals (3 to 8 $\frac{1}{4}$ months) after exposure. However, it would appear that the development to maturity of the worm in some dogs required a longer period than that shown in some cats.

The present experimental skin infection of two cats and two dogs with the advanced third-stage larvae of the worm required a comparatively shorter time before the first finding of eggs in the stools than previously shown through feeding experiments with larvae performed on cats by Prommas and Daengsvang (1937) (6 $\frac{1}{2}$ months and 7 $\frac{1}{2}$ months) and by Miyazaki (1950) (242 and 434 days).

These findings indicate that the definitive host (cats, dogs, etc.) of the parasite may be naturally infected not only by the oral route but also by skin penetration by the advanced third-stage larvae. Unequal rates of development of the worms during migration in the body of the animal are observed. The larvae become immature adults before penetration into the stomach wall where maturity is developed. The period required for the development into adult worms after skin penetration of the larvae in a few cases is somewhat shorter than that required after oral infection. The indication is that skin infection is another possible mode of transmission which could be of significance equal to that of oral infection to the human population. Those persons involved in the handling of animal flesh might be especially vulnerable. In this connection the migration and development of the larval stage after skin penetration in man might be similar to that found in the definitive host. However, this remains to be further studied.

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