Transcutaneous Infection by Gnathostoma spinigerum

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OBJECTIVE: This study is designed to characterize experimental gnathostomiasis induced by the recently discovered transcutaneous route.

DESCRIPTION: Weekly stool examinations, by the formalin–ether concentration method of cats and dogs previously infected by skin penetration with G. spinigerum advanced third–stage larvae, were undertaken to determine the time required for the first ova positive stools (prepatent period) and the period of ova positive stools (patent period) of the infected animals. Quantitative estimates of the numbers of ova in the stools of the positive animals were also made by Stolls' egg counting technique. Autopsies were performed on cats and dogs to examine the migration and development of G. spinigerum larvae in various organs of the animals at various times after being transcutaneously infected with larvae of the worm.

PROGRESS: Observations of the skin penetration of third–stage larvae of G. spinigerum were continued on 7 cats and 7 dogs (see 1969 Annual Report), and the study was expanded to include 2 additional adult cats and 1 adult dog. The results were as follows:

Cat #73 died of unknown causes on 22 July 1969 with the stool still positive for ova (144 + days of patent period). Autopsy showed a small round gastric tumor of about 1.0 cm in diameter containing 2 mature male G. spinigerum measuring 13.0 mm × 1.2 – 1.3 mm and 1 mature male attached to the omentum near the greater curvature of the stomach measuring 14.0 × 1.4 mm. Since the animal had been inoculated with 42 larvae, the rate of recovery of worms was only 7.1%. No female worms were found in the gastro–intestinal tract and had the animal lived, the next stool examination would have been negative for ova. In this case probably many adult males and all females passed out with the stools before death (spontaneous elimination by the cat).

Cat #74 died on 1 April 1969 (probably as a result of vaccination against distemper), 195 days after the initiation of the experiment, and was still in a period of ova negative stools. On necropsy, the animal showed 17 immature male and female G. spinigerum in the diaphragm and anterior chest wall and 9 growing third–stage larvae in the anterior chest wall musculature: abdominal wall, back and the stomach wall. The rate of worm recovery was 56%

Cat #77 first showed ova positive stools on 13 June 1969 or about 227 days after skin infection (prepatent period). It died 28 days following the first ova positive stool, probably due to rupture of a gastric tumor caused by the G. spinigerum adults. On autopsy 46 organisms were found (rate of worm recovery was 54%) of which 30 were mature worms, 14 immatures and 2 advanced third–stage larvae infecting many organs as follows: 12 mature (8 females and 4 males) free in stomach cavity; 9 mature (6

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females, 3 males) located in a gastric tumor measuring about 2.0 cm. in diameter; 7 mature (5 females, 2 males) found attached to the peritoneal fat near the outer surface of a stomach tumor; 2 mature females in the left diaphragm. Fourteen immature worms (12 females, 2 males) were located in the following organs: 9 females attached to peritoneal fat near the lower part of the stomach, 4 (2 females, 2 males) located in the stomach tumor, and 1 female in the intercostal muscles of the lower chest wall musculature and one in the left diaphragm.

Cat #83 showed the first ova positive stool on 2 September 1969, after a prepatent period of 310 days. Stool examination was first negative 89 days later (patent period). The animal is still being kept for further observation.

Cat #84 first showed ova positive stools on July 1969 (a prepatent period of 127 days). The first ova negative stool was found 100 days later (patent beriod). The animal was kept for further observation.

Cat #87 and #89 died during the period of ova negative stools probably as the result of vaccination against distemper on the same date of worm development and the infectivity rates in the animal. On necropsy there were a total of 41 worms recovered with the following details: 37 mature (22 females and 15 males) and 2 immature female G. spinigerum found in a round gastric tumor measuring 2.0 cm. in diameter. In addition there was 1 immature female located in the right lung and 1 immature female attached to the peritoneal tissue near the lower part of the stomach. The worm recovery rate was 63%.

Dog # 2 showed the first G. spinigerun ova positive stools on 22 May 1969 (prepatent period of 247 days after the first skin infection); the first ova negative stool was on 3 February 1970 (patent period of 257 days). The animal was kept for further study.

Dog # 9 showed the first G. spinigerum ova positive stools on 3 June 1969 (prepatent period of 22 days); the period of ova positive stools was 58 days (patent period). The animal was then sacrificed 57 days after the last ova positive stool; autopsy showed no stomach lesion, but there were 9 advanced third—stage larvae measuring $6.0 - 6.8 \times 0.6 - 0.7$ mm found in the muscles of the hind legs (4 larvae), in the intercostal muscles (3 larvae), and in the flesh of the anterior abdominal wall (2 larvae). The worm recovery rate is 4.7%.

Dog # 12 the animal always showed ova negative stools on weekly stool examinations. It was sacrificed on 26 February 1970, 498 days after the skin infection and on autopsy revealed a total of 2 G. spinigerum larvae in the muscles of the back and fore—leg measuring 8.0×0.7 mm and 4.0×0.6 mm (worm recovery rate 2.3%).

Dog # 13 first became ova positive 112 days after the first skin infection or 93 days after the last (prepatent period). The animal then continued showing ova positive stools for more than 329 days up to the date of termination of the study (17 October 1969).

Dog # 14 was sacrificed on 23 July 1969 after 253 days (8 1/2 months) of patency. Autopsy showed a gastric tumor of about 2.0 cm in diameter containing 19 mature worms (9 males and 10 females), and there were 12 mature worms (3 males, 9 females) found freely mixed with the stomach contents. In addition, there was 1 immature worm found in the right side of the diaphragm and another in the lower part of the esophageal wall. The total burden was 33 worms (rate of worm recovery about 49%).

Dog # 18 was given on August 13, 1969, one transcutaneous infection with 64 G. spinigerum larvae in 1 1/2 hours (rate of successful penetration, 100%). The animal up to the end of 31 March (230 days after the skin infection) was still showing ova negative stools. It was kept for further observation.

The result of this experimental investigation on skin penetration of dogs by G. spinigerum larvae is summarized in Table 2.

SUMMARY: Observations of cats and dogs transcutaneously infected with third—stage larvae of G. spinigerum were continued. Prepatant periods in the cats ranged from 60 to 310 days, and the periods of positive stools (patent periods) varied from 89 to more than 197 days. Recovery of inocula as viable worms ranged from 7.0% to 94.4%.

In dogs, the prepatent period was from 96 to 247 days. One dog continued to be negative for 498 days, and at autopsy only 2 larvae were recovered. The period of patency (ova production) ranged from 58 days to 329 days. The worm recovery rate varied from 2.4% to 85%.

There appeared to be a continuous reduction of the number of worms harbored by both dogs and cats as the infections persisted. The recovery rate decreased to 7% from rates as high as 94% obtained from cats 22 days after exposure; the rates decreased to 7% 320 days after exposure. In dogs, 85% could be recovered the day after penetration of the larvae and only 4.7% of the inoculum was found 280 days after exposure.

Table 1. Skin penetration by Gnathostoma spinigerum advanced third-stage larvae in 10 adult domestic cats.

Animal No.	Source of larvae	Date and frequency of infection	No. of larvae penetrated thru the skin and %	Days from skin penetration of larvae to first ova positive stool (prepatent period)	Days from first ova positive stool to first ova negative (patent period)	Autopsy findings			Remarks
						No. of worms recovered and %	Stages of worms recovered	Organs infected by worms	
*Cat 38	white mice & snake-headed fish	13-22/8/68 3 times	53 (62%)	154 +	-	27 (51%) males, females and larvae	immature adult and larvae	stomach abdominal wall chest wall omentum diaphragm	Sacrificed on 14 Jan 69 (day 154 of prepatent period)
Cat 73	white mice dog	5-6/9/68 2 times	42 (93%)	176	144 +	3 males (7.0%)	mature adult	Gastric tumor and omentum	Died of unknown causes with ova positive stool (day 144, on 22 July 69)
Cat 74	snake-headed fish	17/9/68 1 time	46 (90%)	195 +	-	26 males, females and larvae (56%)	immature adult and larvae	stomach diaphragm, costal, abdominal and back muscles, abdominal fat.	Died perhaps of vaccination (on 1 April 69, day 195 of prepatent period)
Cat 77	white mice	29/10/68 1 time	85 (100%)	227	28 +	46 mature and immature and larvae (54%)	mature and immature adults and 1 larva	stomach diaphragm peritoneum muscles of abdomen and chest.	Died during ova positive stools (day 28 of patent period, on 11 July 69)
Cat 83	white mice	30/10/68 1 time	61 (100%)	310	89	-	-	-	Still alive, ova negative stool up to 31 March 70 (118 days after last positive stool)
Cat 84	white mice	25-28/2/69 2 times	44 (66.7%)	127	100	-	-	-	Still alive, ova negative stool up to 31 March 70 (172 days after last positive stool)
Cat 87	white mice	10/3/69 1 time	18 (100%)	22 +	-	17 larvae (94.4%)	larvae	liver, skin, diaphragm abdominal fat	Died perhaps of vaccination on 1 April 69 (day 22 of prepatent period)
Cat 89	white mice	10/3/69 1 time	45 (75%)	22 +	-	38 larvae (84.4%)	larvae	liver abdomenal muscle abdominal fat	Died perhaps of vaccination on 1 April 69 (day 22 of prepatent period)
Cat 91	white mice	17/7/69 1 time	59 (93%)	60	197 +	-	-	-	Still alive, ova positive stool up to 31 March 70 (day 197 of patent period)
Cat 97	white mice	7/8/69 1 time	10 (100%)	60 +	-	7 larvae (70%)	larvae	liver	Died on 6 Oct 69 (day 60 of prepatent period)

*Reported in 1969 Annual Report.

Table 2. Skin penetration by Gnathostoma spinigerum advanced third-stage larvae in 9 adult domestic dogs.

Animal No.	Source of larvae	Date and frequency of infection	No. of larvae penetrated thru the skin and %	Days from skin penetration of larvae to first ova positive stool (prepatent period)	Days from first ova positive stool to first ova negative (patent period)	Autopsy findings			Remarks
						No.of worms recovered and %	Stages of worms recovered	Organs infected by worms	
Dog 1	white mice	15/10/68 1 time	65 (79.3%)	231	31 +	41 mature males & females (63 %)	mature adult	stomach lung omentum	Sacrificed on 3 July 69 with ova positive stools (day 31 of patent period)
Dog 2	snake & snake-headed fish	17-23/9 /68 2 times	76 (100%)	247 (from first skin infection)	257	-	-	-	Still alive, ova negative stools up to 31 March 70 (56 days after last positive stool)
Dog 9	white mice	24/10-4/11/ 68 2 times	192 (81.4%)	222 (from first skin infection)	58	9 larvae (4.7%)	larvae	hind leg muscle costal muscle abdominal muscle, abdominal fat	Sacrificed with ova negative stools, 26 Sept69 (57 days after last positive stool)
Dog 10	white mice & snake	28/10 - 12/11/68 2 times	119 (69.6%)	234 (from first skin infection)	228	-	-	-	Still alive,ova negative stools up to 31 March 70 (57 days after last positive stool)
*Dog 11	white mice	4/9/68 1 time	33 (97.1%)	1 +	-	28 larvae (85.0%)	larvae	skin abdominal flesh.	Died on 5 Sept 68 with ova negative stool (day 1 of prepatent period)
Dog 12	white mice	16/10/68 1 time	88 (96.7%)	498 +	-	2 larvae (2.3%)	larvae	dorsal muscle fore-leg muscle	Sacrificed on 26 Feb 70 with ova negative stool (day 498 of prepatent period)
Dog 13	white mice	2-21/8/ 68 3 times	64 (38.6%)	112 (from first skin infection)	329 +	-	-	-	Discharged on 17 Oct 69 from the study for being uncontrollable.
Dog 14	white mice	8-20/8/ 68 2 times	68 (46.2%)	96 (from first skin infection)	253 +	33 immature females, mature males & mature females (48.5%)	immature females, mature males mature females	stomach diaphragm esophageal wall.	Sacrificed on 23 July 69 during ova positive stools (day 253 of patent period)
Dog 18	white mice	13/8/69 1 time	64 (100%)	230 +	-	-	-	-	Still alive, ova negative stool up to 31 March 70 (day 230 of prepatent period)

*Reported in 1969 Annual Report.