

Experimental Detection of Gnathostomiasis Antibody
by Gel-Diffusion

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OBJECTIVE : To develop a gel-diffusion test to detect gnathostomal antibody in laboratory animals and humans and to document the immune response of these hosts to active infections with this nematode.

BACKGROUND : Methods of transmission and reservoir hosts for *Gnathostoma spinigerum* have been well documented (1). Although some work has been done with this nematode using precipitin ring tests and the Sarlis phenomenon, methods of detecting gnathostomiasis have not been completely worked out (2, 3). In the present study, detection of an anti-gnathostome antibody in cats and rats artificially infected with *G. spinigerum* is reported.

DESCRIPTION : Two studies were conducted to determine whether a suitable *G. spinigerum* antigen could be successfully extracted from advanced third-stage larvae, and to see if this antigen could be used satisfactorily in detecting antibody in artificially infected rats and cats.

Parasite Antigen Source : Material used to prepare the antigen was obtained from experimentally infected mice harboring *G. spinigerum* third-stage larvae. The mice were exsanguinated or allowed to die and the larvae dissected immediately from the tissues and placed in phosphate buffered saline (PBS), pH 7.5. This material was freeze-dried and stored until needed for antigen preparation.

Preparation of the Antigen : Freeze-dried *G. spinigerum* third-stage larvae were weighed out in 500 gm portions, placed in a glass homogenizer and de-lipidized with absolute alcohol and anhydrous ether. Following de-lipidization the homogenate was extracted by constant stirring at 2-5°C in PBS (pH 7.5) overnight. The extracted material was then centrifuged at 15,000 rpm for 15 min and the resulting supernate used as the antigen.

Double-Diffusion Test : The double-diffusion test was conducted by the method of Ouchterlony (4). Hyperimmune serum for use as a positive control was obtained from rabbits (5, 6) immunized with the antigen extracted from advanced third-stage *G. spinigerum* larvae. Equal portions of antigen and rat or cat serum were then added to the reaction chambers and allowed to react for 2-5 days before recording results.

EXPERIMENT NUMBER ONE :

PROGRESS : Five hundred rats were each infected orally with ten *G. spinigerum* third-stage larvae obtained from exsanguinated donor mice. Experimental rats were killed in pairs every two days and the serum tested for anti-gnathostome antibody using immuno-diffusion with the advanced third-stage larval extract as the antigen.

Anti-gnathostome antibody was detected as early as 13 days (Table 1) after infection and could still be detected as long as 151 days after infection. In rodents *G. spinigerum* rarely develops past the third-stage larval phase. Since the third-stage larvae were used as the antigen in this experiment, the positive reactions observed in the gel-diffusion test were presumably due to antibodies developed against *G. spinigerum* larval forms.

Table 1. The immune response of experimentally infected rats harboring *G. spinigerum* third stage larvae

DAYS AFTER INFECTION WITH <i>G. SPINIGERUM</i>																										
DAYS	0		11		13		15		17		19		21		23		25		27		29		31		33	
RAT #	-	-	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
RESULTS	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	NS	NS	-	+	+	-	+	+
DAYS	35		37		39		41		43		45		47		49		51		53		55		57		59	
RAT #	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
RESULTS	-	-	-	-	+	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	NS	NS
DAYS	61		63		65		67		69		71		73		75		77		79		81		83		85	
RAT #	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
RESULTS	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
DAYS	87		89		91		93		95		97		99		101		103		105		107		109		111	
RAT #	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112
RESULTS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DAYS	113		115		117		119		121		123		125		127		129		135		141		145		147	
RAT #	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	135	136	141	142	145	146	147	148
RESULTS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
DAYS	149		151																							
RAT #	149	150	151	152																						
RESULTS	+	-	+	+																						

N.S. = NO SAMPLE

Table 2. Immuno-Diffusion Results on *G. Spinigerum* Infected Cats

DATE	CAT # 120 (inf. Apr and May 72)	CAT # 132 (inf. Feb and Aug 73)	CAT # 135 (inf. Jul 73)
28 Sep 73	Positive	Positive	No specimen
5 Oct 73	Positive	Positive	Positive
12 Oct 73	Positive	Positive	Positive
19 Oct 73	Positive	Positive	Positive
26 Oct 73	Positive	Positive	Positive
2 Nov 73	Positive	Positive	Negative
9 Nov 73	Positive	Positive	Negative
16 Nov 73	Positive	Positive	Positive
22 Nov 73	Positive	Positive	Negative
30 Nov 73	Negative	Positive	Negative
14 Dec 73	Negative	No specimen	Negative
21 Dec 73	Negative	Negative	Negative
28 Dec 73	Negative	Negative	Negative
4 Jan 74	Negative	Negative	Negative
11 Jan 74	Negative	Negative	Negative
18 Jan 74	Negative	Negative	Negative
25 Jan 74	Negative	Negative	Negative
4 Feb 74	Negative	Negative	Negative
8 Feb 74	Negative	Negative	Negative
15 Feb 74	Negative	Negative	Negative
22 Feb 74	Negative	Negative	Died
1 Mar 74	Negative	Negative	
7 Mar 74	Killed		

Table 3. Necropsy Results on *G. Spinigerum* Infected Cats

CAT #	Total Larvae found	Right Front leg	Left Front leg	Right hind leg	Left hind leg	Abdomen	Back	Stomach	Diaphragm
		Fat Muscle	Fat Muscle	Fat Muscle	Fat Muscle	Fat Muscle	Fat Muscle	Tumor	Muscle
120	30	— 4	— 3	1 5	2 7	5 1	1 1	—	—
135	45	— —	— —	— —	— —	— 7*	— —	32*	6*

* All Immature Adults

Comparative studies using sera from rabbits immunized with crude antigen and sera from actively infected rats indicated that antibodies were present against the third-stage larval antigen in both, although specific serological identity has yet to be proven (Fig. 1, 3). In one rabbit (Number 5) a minimum of three distinct precipitin bands could be detected while in the rat model only two have been detected thus far. In the rat, this second band first appeared 71 days after infection and may be due to antibody against antigen released during the worms' maturation.

Up to now it has not been conclusively demonstrated that the antibody detectable in the rat system is identical to that seen in the control rabbit but present evidence suggests they are the same.

EXPERIMENT NUMBER TWO:

PROGRESS: Three adult cats (120, 132 and 135) were selected for the study. Cats 120 and 132 had been infected previously for different experiments in April and May 1972 and in February and August 1973 and had both previously shown *G. spinigerum* ova in their stools. Cat 135 had never been used before and served as a positive control. Cat 135 was infected on 17 July 1973 for the first time with 205 third-stage larvae, 155 larvae by active skin penetration and 50 by oral administration. Weekly bleedings on all the cats except 135 were commenced on 28 September 1973 and have been continued to the present (Table 2). Cat 120 which had already been infected for almost 1 year and 10 months demonstrated positive double-diffusion tests on the first day of bleeding. Similarly, cats 132 and 135 which had been more recently infected showed positive results (Table 2). Positive results were obtained in cat 132 and 120 until November 1973. Cat 135 first became negative on 2 November 1973 although one more positive immuno-diffusion test was obtained on 16 November 1973. This cat then became negative and remained so until his death on 15 February 1974. At necropsy of cat 135 we found only immature adult stages of *G. spinigerum* (Table 3). *G. spinigerum* ova were never recovered from the stools of cat 135, ruling out the possibility of adult nematodes in the stomach.

Cats 120 and 132, which were previously infected, produced results similar to cat 135 except that they were positive in the gel-diffusion test for a longer period of time (Table 2). Cat 120 was sacrificed to determine if it also harbored the immature adult or the adult stages of this parasite because maturational stage differences in this case may have been responsible for the negative gel-diffusion tests observed. At necropsy all the worms found were encysted advanced third-stage larval forms, even though 1 year and 10 months had elapsed since last exposure to infection (Table 3). This development is noteworthy, since, once the definitive host becomes infected, the advanced third-stage larvae usually develop into immature adults within few months. All the larvae found in cat 120 had become encysted

PRECIPITIN REACTION USING RABBIT, RAT, AND CAT ANTISERA
 AGAINST G. SPINIGERUM THIRD-STAGE LARVAL ANTIGEN

Fig. 1

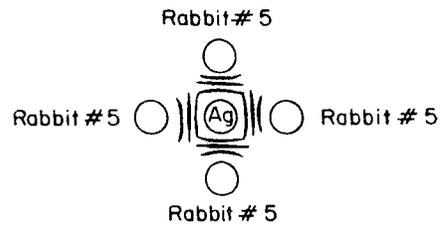


Fig. 2

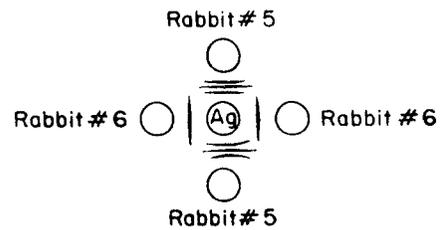


Fig. 3

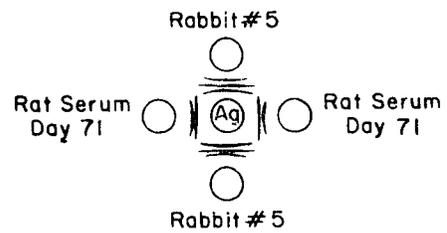
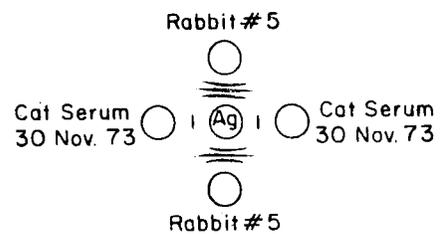


Fig. 4



and were not actively migrating. To date, cat 132 remains negative in gel-diffusion tests, although he has been positive for gnathostome eggs in the stool since Feb 1974

DISCUSSION: The double-diffusion test appears to be helpful in detecting third-stage larval infections in the rat host. The nature of the specific antibody being detected is presently unknown. The ability to detect antibody against the third-stage larvae of this parasite for as long as 151 days in the rat is interesting. The technique may be applicable in human infections where the third-stage larval parasite is present. The negative double-diffusion test results in cat 135 after November 1973 may have been due to differences in the antigenic stimulus being presented after the advanced third-stage larvae molted into immature adult forms. A definitive answer as to whether there are qualitative antigenic differences between the different stages of this parasite could be obtained by further study. Negative gel-diffusion results in cat 120 after November 1973 may have been because the encysted larvae were no longer providing sufficient stimulus for the level of precipitating antibody to be detectable by immuno-diffusion. This theory is supported by the fact that cats 121, 122, and 123, infected in July 1973 for purposes other than our experiment, showed positive gel-diffusion tests even after cats 135, 132, and 120 had become negative. Cat 122 at necropsy showed actively migrating larvae. In order to detect active third-stage larval infections of *G. spinigerum* by gel-diffusion, actively migrating larvae and stage specific antigens may have to be present. Studies are now being conducted in this area. Comparative studies with immune cat serum and immune positive control rabbit serum have shown results similar to the rats; however, serological identity of the anti-gnathostome antibody in both systems to a common antigen has not yet been demonstrated.

SUMMARY: An antigen extracted from advanced third-stage *G. spinigerum* larvae was used to experimentally detect anti-gnathostome antibody in artificially infected rats and cats. In rats, precipitating antibody could be detected as early as 13 days after infection and as long as 151 days after infection. Studies with the gel-diffusion test using third-stage larval antigen could detect the larval migratory phases of this nematode in the definitive host, but, when this parasite molted into an adult or encysted as a third-stage larva, detection by gel-diffusion was no longer possible. The immuno-diffusion test using third-stage larval antigen could be useful in detecting human infections where the third-stage larval parasite is present. Larval migratory periods of up to 10 years or more have been reported in humans (5). Studies on the cross-reactivity of this antigen with other helminthic infections are also being conducted and studies of immunological detection of gnathostome infection in primates are being planned.

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