CIRCUMOVAL AND LARVAL MICROPRECIPITATION REACTIONS IN EXPERIMENTAL AND HUMAN GNATHOSTOMIASIS

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INTRODUCTION

Circumoval precipitation test (COPT) has been widely used for clinical diagnosis and evaluation of chemotherapeutic cure of schistosomiasis in many endemic areas (Oliver-Gonzalez, 1954; Hillyer *et al.*, 1981; Kamiya, 1983). The technical simplicity and a long shelf-life of the lyophilized eggs used in the test has made it also the most suitable immunodiagnostic assay for mass screening of the infection in epidemiological work or other kinds of field study. Recently, the technique has been adapted successfully for serodiagnosis of opisthorchiasis (Flavell, 1981). Another simple method namely the larval microprecipitation test (LMP), although with a disadvantage due to the requirement of living larvae, has been in use for diagnoses of many nematode infections including toxocariasis, trichinosis, among others (Negru *et al.*, 1972; Preisshogen and Lamina, 1977; Kamiya, 1982). In case of gnathostomiasis, immunological methods which have been employed are not only non-specific but also insensitive and require complicated laboratory procedures. Our studies reported herein, is, therefore, to evaluate the value of COPT and LMP in diagnoses of experimental and human gnathostomiasis using various developmental stages of the parasite against various sources of immune sera.

MATERIALS AND METHODS

Sera: The sera used in this study were obtained from either experimentally *G. spinigerum* infected mice, rats, cats, naturally infected humans or from their parasite-free control counterparts, respectively.

Albino mice: Two groups of 50 albino Swiss mice aged two months old obtained from the animal house of the Faculty of Tropical Medicine were orally infected individually with either cyclops containing about 30 early third-stage larvae (EL3) of *G. spinigerum* or 5 advanced third-stage larvae (AL3) obtained from laboratory infected mice. Five mice of each group were bled before they were killed on every consecutive week for ten weeks. The number of worm recovered from each mouse were counted while their sera were kept at $-20\ ^{\circ}$ C before use in the COPT and LMP.

White rats: Twenty, two-month-old albino Wistar rats of both sexes were individually

infected with 20 AL3. Two animals were bled and killed every week for serum collection and worm recovery, respectively.

Cats: Five healthy parasite-free young adult cats were infected with 44, 45, 60, 74 or 75 AL3, respectively. Serum samples were monthly collected for ten consecutive months and were kept at −20 ° C. All cats were then operated upon, adult worms were collected and counted.

Human sera: Two proven (immature male *G. spinigerum* recovered from surgery of a hip swelling, and peritoneun during appendicectomy) and eight presumptive cases (history of eating raw meat and appearance of migratory swelling on the skin) of human gnathostomiasis were used in this study.

Antisera to heterologous parasitic infections: Laboratory animals were infected with *Trichinella spiralis* (3 mice), Japanese strain of *Schistosoma japonicum* (6 mice), *Paragonimus heterotremus* (2 cats), *Angiostorongylus cantonensis* (2 rats) and *Opisthorchis viverrini* (4 hamsters). Sera were collected from these infected animals at 46, 72, 80, 139 and 49 days after infections, respectively.

Developmental stages of *G. spinigerum*: Unembryonated ova, embryonated ova, first stage larvae (L1) and advanced third-stage larvae (AL3) either living or air-dried preparations (Kamiya, 1983; Oku and Kamiya, 1984) were used as the source of antigens.

Unembryonated eggs were obtained from the uteri of mature female worms which were collected from infected cats. Some of them were subjected to *in vitro* culture for seven days and nine days to yield embryonated eggs and L1, respectively. The preparations were thoroughly washed with normal saline and distilled water. A number of these suspended ova or L1 were then adjusted to give about one hundred when few drops were applied onto a clean slide. They were air-dried overnight at room temperature and were then kept at −20 °C. AL3 were obtained from mice which had been infected with EL3 for 3 weeks. They were washed, air-dried and kept at −20 ° C.

COPT and LMP: One drop of serum was used to incubate with the prepared slide of ova or L1 while few drops of it were applied on a welled slide which contained 5 AL3. The preparation was covered with a cover slip, sealed and incubated at 37 ° C. The reaction was then examined under a microscope at 10 X magnification within 72 hours.

RESULTS

The LMP reaction between antisera and AL3 of *G. spinigerum* are shown in Figs. 1−8, while the COPT showed negative results since no precipitates appeared when the homologous sera were tested against either fresh or air-dried unembryonated eggs, embryonated eggs or L1. Positive LMP results were clearly seen when the air-dried AL3 and some homologous sera were used in the reaction but less so with the living larvae owing to their active motion. (Figs. 2 to 8).

The precipitates formed on the larvae were roughly classified into two types, the membranous and the filamentous forms. The filamentous precipitates were distributed randomly on the surface of the AL3 (Figs. 3, 6 and 8), while the membranous precipitates were formed around the esophageal region (Figs. 2, 5 and 7). The degree of precipitation were arbitrarily classified as weak (1+), moderate (2+) and strong (3+) reactions according to the extent and amount of the

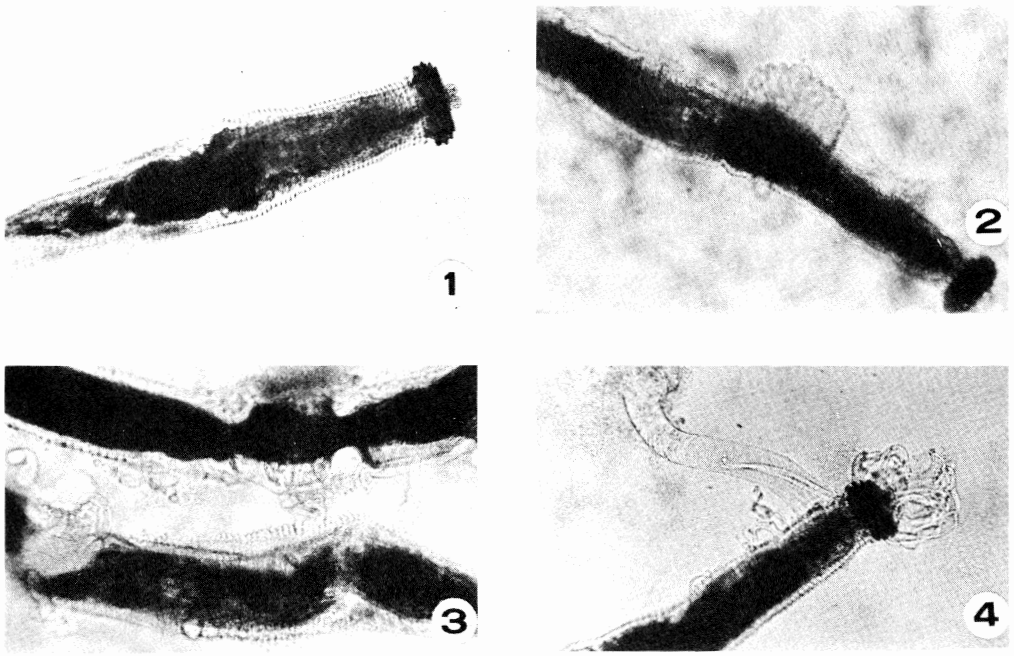
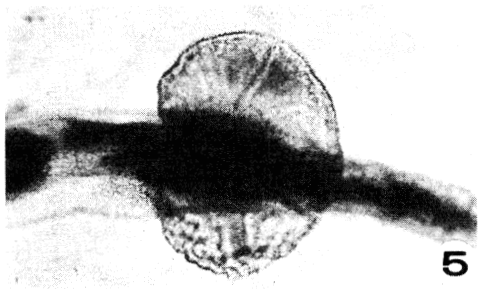


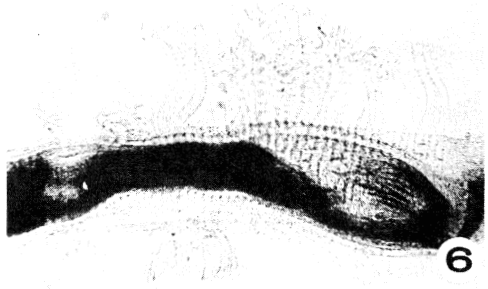
Fig. 1–Negative LMP reaction: normal AL3 after incubation for 48 hours with serum of control non-infected cat.
Fig. 2–Weak LMP reaction: delicate membranous precipitate at the excretory pore of AL3 after incubation for 48 hours with parasitologically confirmed serum.
Fig. 3–Weak LMP reaction: filamentous precipitates on the cuticular surface of AL3 with serum of a presumptive gnathostomiasis case.
Fig. 4–Filamentous precipitates at the head bulb and neck of AL3. A large band of precipitate extends from the neck of the worm after incubation for 48 hours with serum of cat infected with 75 AL3 at four months post-infection.

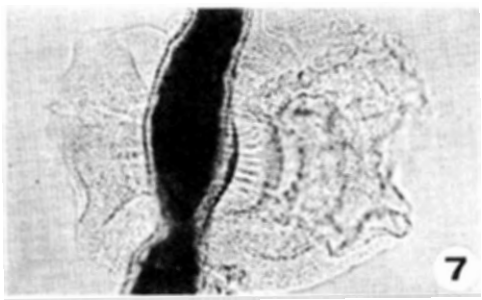
precipitates on the parasite surface.

Weak LMP reaction was demonstrated when only one out of the two parasitologically confirmed gnathostomiasis sera (Fig. 2) and two out of eight presumptive gnathostomiasis sera were used in the test (Fig. 3). No LMP occurred when all immune sera obtained from animals infected with *T. spiralis*, *S. japonicum*, *P. heterotremus*, *A. cantonensis* and *O. viverrini* were incubated with the larvae.

Encystation of the larvae occurred within the fourth week post-infection and was completed within the eighth week in mice infected with EL3. However, precipitating antibodies giving positive LMP reaction were detected in these infected mice since the second week and remained so thereafter. In mice infected with AL3, encystation occurred in the second week and was completed in the fifth week. No antibodies yielding tegumental precipitates were found until the seventh week of infection in these mice. In rats infected with AL3, encystation started in the fourth week and was completed within the following week. Weak to moderate LMP reactions, mostly membranous type, were







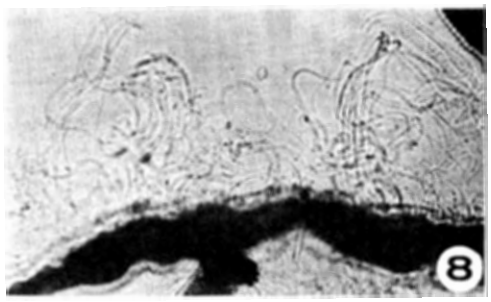


Fig. 5–Moderate membranous precipitin reaction formed around esophagus of AL3 with serum of mice infected for 4 weeks with EL3.

Fig. 6–Moderate filamentous precipitin reaction formed on AL3 with serum of a cat infected with 45 AL3, at 5 months post-infection.

Fig. 7–Strong precipitin reaction (membranous type) around the esophagus of the larva with serum of cat infected for 2 months with 44 AL3.

Fig. 8–Strong precipitin reaction (filamentous type) extend from the integument of AL3 with serum of a cat infected with 44 AL3, one month post-infection.

observed when AL3 were incubated with the rat sera taken after the first week of infection.

The infectivity of the larvae in cats infected with 44, 45, 60, 74 and 75 AL3 were 61.4, 28.3, 21.7, 33.8 and 31.1%, respectively. Considerable moderate, very weak and weak LMP reactions were seen in antisera of cats infected with 44, 45 and 74 AL3, respectively while strong reactions were observed when antisera of the other two infected cats were used in the test (Figs. 7 and 8).

DISCUSSION

Precipitin formations around the mouth, vulva and anus of AL3 after incubation with rabbit hyperimmune sera (Sarles' phenomenon) has been demonstrated by Furuno since 1959. Reproducibility of such finding is observed in the present study using immune sera of mice, rats and cats infected with either early third-stage (EL3) or advanced third-stage (AL3) larvae. The antigens responsible for the reaction have been shown to be excretory-secretory products which are

enzymes (Rattanasiriwilai *et al.*, 1985). These enzymes seem to present in all stages of the parasite development at the levels which are high enough to evoke the precipitating antibody responses in the hosts (mice, rats and cats). However, the so-produced precipitating antibodies gave positive *in vitro* antigen-antibody reaction only with the AL3 which indicated that the epitopes are located exteriorly only on that parasite development stage. This LMP is not only found to be stage specific but also be parasite specific as no reaction occurred when heterologous sera were incubated with the AL3. However, sensitivity of the test in human gnathostomiasis needs to be considered if the assay is going to be used as screening test since only one of the two parasitologically confirmed cases gave positive yet weak reaction.

SUMMARY

Mice, rats and cats were infected either orally or percutaneously with a number of early or advanced third-stage larvae (EL3 or AL3, respectively) of *G. spinigerum*. Sera obtained from these infected animals and 10 human gnathostomiasis cases were tested against various developmental stages of the parasite which were prepared and used while being alive (fresh) or dead (air-dried) for the circumoval and larval microprecipitation (COP and LMP) reactions.

No precipitin reactions were observed in all sera tested against unembryonated eggs, embryonated eggs and first stage larvae neither air-dried nor fresh preparations. Sera were merely reactive giving various degrees of membranous or filamentous precipitates against the air-dried preparation of AL3.

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