A SIMPLE METHOD OF ARTIFICIAL HATCHING OF *TOXOCARA CANIS IN VITRO* AND PREPARATION OF EXCRETORY-SECRETORY ANTIGEN

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HUMAN infection with second-stage larvae of the canine nematode, *Toxocara canis*, was first reported by Beavear *et al*[1]. The second-stage larva hatches out from the ingested egg in the alimentary tract of the host, migrates through the intestine and invades the other viscera. It is referred to as toxocaral visceral larva migrans (VLM) as it does not develop into adult worm in unnatural hosts like man and mouse.

Diagnosis of VLM by direct demonstration of the larvae is often impractical and serological tests are more suitable. Crude extracts of adult worms, embryonated eggs or hatched larvae have been employed as antigens to detect antibodies. These have been found to give variable results and have drawbacks due to the cross-reacting somatic antigens with other nematodes[2,3].

Fife[4] has reviewed the immunodiagnosis of parasitic diseases and has stressed the potential role of excretory-secretory (ES) antigen of the parasite in sero-diagnosis. Some workers[5,6] have reported *in vitro* hatching of *T. canis* larvae for cultivation and maintenance. They prepared ES antigen from the pooled spent culture fluid by ultrafiltration with Amicon HICPO or UM_2 ultrafilters. These workers have used the method of Fairbairn[7] originally used for *Ascaris lumbricoides* larval hatching. It required hatching fluid containing reducing substances and other chemicals in the presence of $N_2 + CO_2$ atmosphere (95% + 5%); the viable larvae were collected by the use of Baermann apparatus. These methods of artificial hatching of *T. canis* larvae and collection of viable larvae by Baermann apparatus are cumbersome.

Therefore, we have developed a simpler alternative method for artificial hatching of *T. canis* larvae *in vitro* and preparation of ES antigen by ammonium sulphate precipitation from the spent culture fluid.

Adult female *T. canis* worms were obtained from 3–6 months old stray pups at necropsy. The eggs collected after dissection of the worms were kept in 0.1 NH_2SO_4 at room temperature (24–27°C) for about 3–4 weeks. The embryonated eggs were washed free of H_2SO_4 with normal saline and were treated with 10% sodium hypochlorite solution for 30 min at 37°C. The decoated eggs were washed with normal saline to remove the traces of hypochlorite. These were transferred into a sterile flask containing Eagle's minimum essential medium with Earle's based salts and having (pH 6.5) antibiotics (penicillin, streptomycin and mycostatin). The flask was kept on a magnetic stirrer with a magnetic bar and the stirring was continued for about $2\frac{1}{2}$ hr at 30–35°C until 95–98% of the larvae had hatched. Upto 99% of the hatched larvae were found viable and active. During stirring, the pH of the medium was maintained at 6.4–6.5 by bubbling CO_2 gas inside the flask. The suspension was filtered aseptically through sterilized loose cotton-plug fitted inside the pasteur pipette (with broken tip) and kept inside the screw-capped tube (15 × 150 mm) (figure 1). The tubes were kept in CO_2 incubator at 37°C for 24 hr. Larvae migrated out of the plug and settled at the bottom, whereas the debris and the non-viable larvae got entangled inside the plug. Next day, the pasteur pipette was removed and the larvae were allowed to settle for 30 min. The medium was changed and about 5×10^3 larvae (figure 2) were resuspended in 2.5 ml of fresh medium (MEM with antibiotics pH 6.5) distributed in the screw-capped tubes. The cultures were kept at 37°C in a roller-drum rotating at a speed of 6 revolutions/hr. The pH of the cultures was adjusted to 6.4–6.5 by keeping in CO_2 incubator at 37°C for 24 hr.

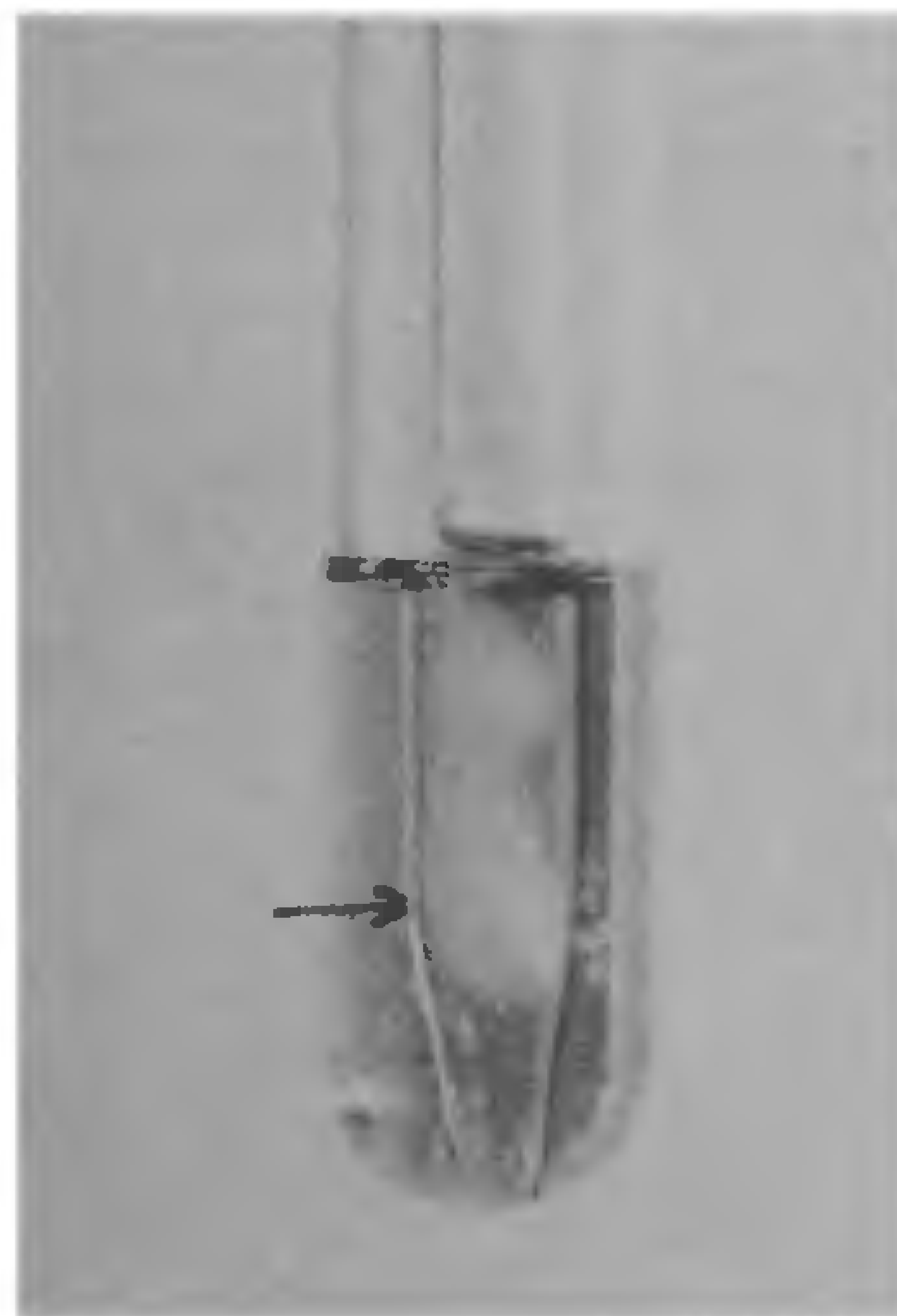
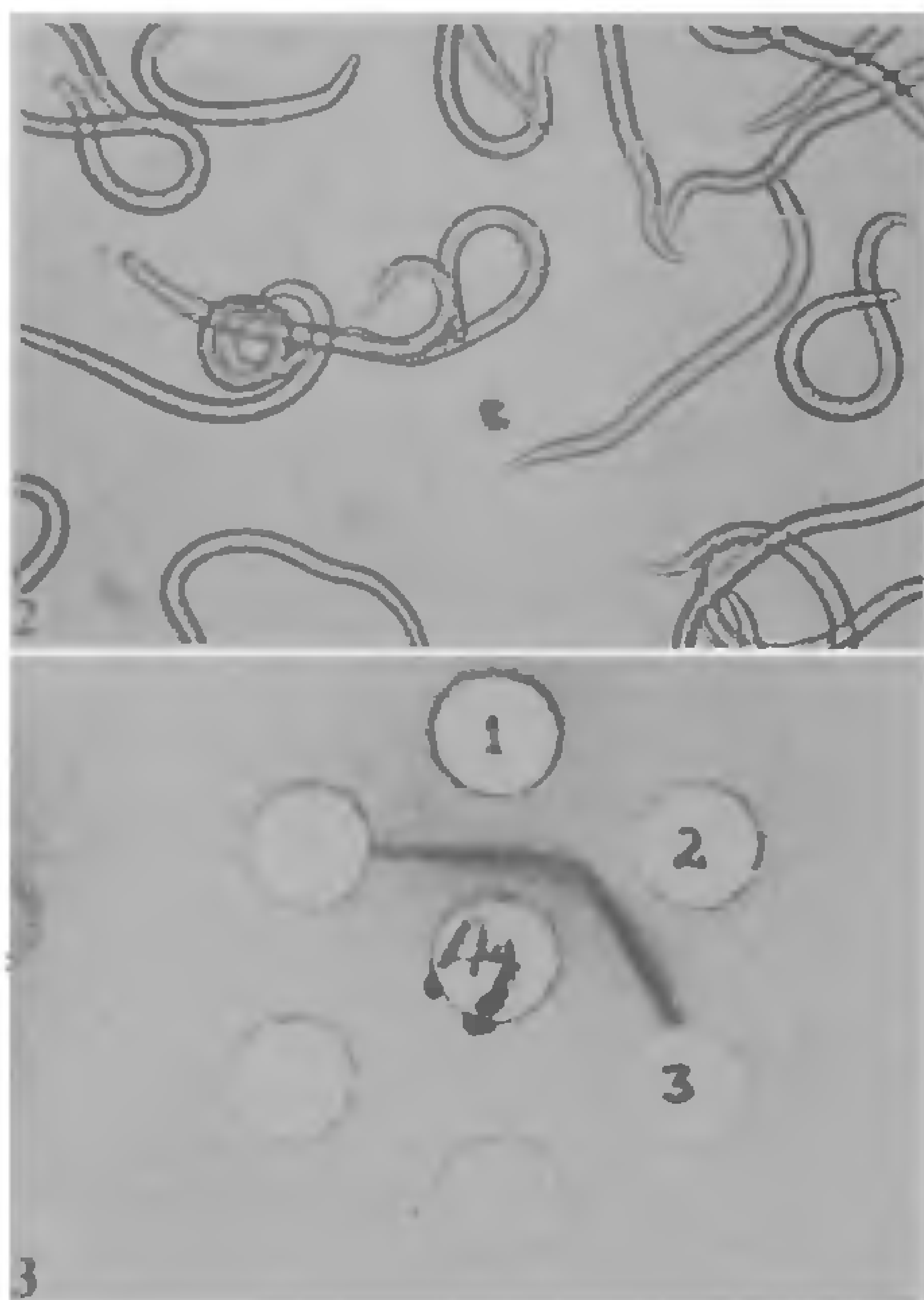


Figure 1. Pasteur pipette containing loose cotton-plug (→ mark), kept inside the tube.



Figures 2 & 3. 2. Hatched and viable *T. canis* larvae. **3.** AGD test showing the precipitin lines centre well (Ag)—ES antigen; 1 and 2 —*T. canis* infected mice sera and 3 —Normal control (uninfected) mouse serum.

The medium was changed every 15 days and the spent pooled medium was stored at −20°C. This spent medium after filtration through millipore filter (0.45 μm), was precipitated with saturated ammonium sulphate solution. The precipitate was removed by centrifugation and was dissolved and dialyzed against normal saline. The final volume was 1/20th of the original fluid. The antigen was checked in agar-gel diffusion (AGD) test[8], which gave precipitin lines against the sera of *T. canis* infected mice (figure 3). The control antigen prepared similarly from the uninfected medium did not show any reaction against *T. canis* infected or normal mouse sera. Twelve Swiss mice were infected orally with *T. canis* larvae (600–700 larvae/mouse) and the sera collected from the 67th to 314th post-infection day, were tested for antibodies in AGD test against the ES antigen. Sera from 7 non-infected (control) mice of the same age and sex were also included. Eleven out of the 12 sera from *T. canis* infected mice reacted with the antigen while all the 7 sera from the control mice were negative.

Employing this method, hatching of *T. canis* larvae was successfully tried seven times in the laboratory. The ES antigen was prepared twice and yielded good reaction against *T. canis* infected mouse sera in the AGD test.

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EFFECT OF MALATHION ON BLOOD GLUCOSE, LIVERGLYCOGEN, PLASMA CORTICOSTERONE AND ELECTROLYTES CONCENTRATIONS AND EOSINOPHIL COUNT IN ADRENALECTOMISED RATS

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ORGANOPHOSPHATE insecticides are known to cause hyperglycaemia and also the increased liver glycogen[1,2]. Previous studies with malathion in this laboratory have shown that when it was administered