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A METHOD OF PREPARING STUDIES OF *TRICHINELLA SPIRALIS* OWEN.

T. T. JOB AND DAYTON STONER.

This work was first attempted with the idea in mind of securing an adequate supply of laboratory material of *Trichinella spiralis* for classes in invertebrate Zoology at the State University of Iowa.

It is the too general belief that such studies are difficult to secure and it is to dispel this idea, in part, that this paper is offered. In fact the comparative ease with which one may secure a presentable series showing the development, growth, migration, encystment, etc., of this worm, affords an unusually good opportunity for illustrating the interesting phenomenon of typical parasitic life.

Since it is often rather difficult to secure trichinized meat from the local shops, the material for the following studies was obtained by addressing the Chief of the United States Bureau of Animal Industry at Washington, D. C. This meat contained the worms in the encysted stage and in suitable condition for transferring to another host where they might live and reproduce.

A part of the trichinized pork was fed to four young white rats which were kept confined in a separate cage. After having eaten of this pork the rats were again given their usual diet.

THE TRICHINELLAE AND HOST.

Host No. 1.—Five days after feeding the encysted *Trichinellæ* the first subject was killed. Various openings were made at different levels in the stomach and intestine and the digestive content together with scrapings from the mucosa were examined under the dissecting microscope in 5 per cent formalin.

Free worms were found only in the intestinal content and the mucosa of the upper ileum. Sections of this portion of the intestine were preserved in 10 per cent formalin.

Host No. 2.—Nine days after feeding, a second subject was killed. The procedure was as in No. 1. In addition, an examination of several blood smears from the superior mesenteric vein and the heart was made. Only one young *Trichinella* was found in the smears and that in the blood from the heart. The embryos at this stage were developed to such an extent that they could readily be seen in the body cavity of the female.

Again sections of the upper ileum were preserved in 10 per cent formalin.

Host No. 3.—Fourteen days after feeding, the third subject was killed. The method was as above. Blood smears were negative. The *Trichinellæ* were found a little further down in the ileum and were much larger than in the nine day stage.

Host No. 4.—The fourth subject was to have been killed twenty-one days after feeding, but it died of trichinosis on the night of the twentieth day.

On examination, free intestinal *Trichinellæ* were found in the middle ileum. The muscles surrounding the abdominal cavity, diaphragm, internal and external oblique, transversalis and psoas, as well as the extensor muscles of the hind legs showed *Trichinellæ* in the *migratory* and *resting* stages. A considerable number were found in these muscles but the masseter muscles showed the various stages even better and more abundantly.

In this stage the entire body of the host was preserved in 10 per cent formalin.

METHOD OF PREPARATION.

The material was handled in watch glasses with pipettes. First, the preserved material was washed thoroughly with distilled water. This not only removes the formalin but separates the *Trichinellæ* from the other material so that the worms may be collected in a pipette and transferred to the next dish. The staining and dehydration were carried on in the same dish so as not to injure or lose the specimens, the different fluids being added and drawn off with the pipette.

Killing and Fixing.—Ten per cent formalin was used in all cases to kill and fix the tissues and *Trichinellæ*. Carnoy's solution may be used with equally good results.

Staining.—Delafield's hematoxylin and erythrosin, orange G, methyl green, borax carmine and iron hematoxylin (Heiden-

hain) were all tried. Iron hematoxylin seemed to give the best results with borax carmine next. Where iron hematoxylin is used, care must be taken to remove all the surplus mordant or a precipitate will occur on addition of the hematoxylin thus vitiating the results. The borax carmine has the advantage in ease of handling.

Clearing—Experiments with xylol, oil of bergamot, chloroform and turpentine showed that all these clearing agents shriveled the specimens. As a matter of fact clearing is not at all necessary.

Mounting.—Specimens mounted in balsam were shriveled just as when treated with a clearing agent so glycerine was used as the mounting medium. The permanent mounts were ringed with lacquer or thick balsam.

It is, perhaps, needless to suggest that great caution be observed in regard to cleaning cages in which hosts are kept, means of disposition of their bodies and general cleanliness in handling specimens.

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