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A SIMPLE AND EFFECTIVE METHOD OF DETECTING TRICHINA IN SAUSAGE

C. S. LINTON

Trichinosis is not a common disease in the United States on account of present day methods of handling meats and the rather universal habit of eating meat only after being well cooked. Occasionally, however, we find people partaking of raw or poorly cooked sausage. An outbreak of the disease resulting from the eating of raw "summer sausage" and involving five or six families and ten or more individuals occurred at Bridgewater, Iowa, last February. Several of the cases proved fatal.

The following description of one of the most severe cases was furnished by Dr. A. Weaver of Cumberland.

"Gastro-intestinal irritation, diarrhoea, weakness, nausea, loss of appetite and vomiting for a week. Then after a lull of 8-10 days, swelling of eyes, soreness of all muscles, typhoid-like symptoms (Widal negative), diazo reaction in urine, five percent of urea, loss of reflexes, difficulty in swallowing, some swelling of the body in general, all muscles sore on compression. There was no joint involvement and finally an eosinophilia of 70% on several counts."

Although the high eosinophilia is quite constant and very characteristic the good clinician desires corroboration of his diagnosis by finding the parasites and wishes to prevent further trouble by detecting the source of the infection. Sausage is eaten raw more commonly than other meats and is less likely to be well cooked. It is, therefore, probably the most common source of infection and at the same time the most difficult kind of material on which to make a satisfactory laboratory examination. The fact that it is always intimately mixed with fatty tissue adds to the difficulties.

It is necessary in this as in other laboratory examinations to have a suitable specimen before a satisfactory examination can be made. Trichina are not evenly distributed in the muscular tissue, and when the carcass is trimmed, and the portions to be made into sausage selected, it is not difficult to understand how much of the sausage may be quite free of the trichina. In taking a sample for submission to the laboratory three to four sections 4-6 inches long,

or an equivalent, should be carefully selected. The small sample sent in a vial or a 1-2 ounce bottle is never satisfactory.

It is well known that the muscles of the diaphragm are usually heavily infected with *Trichina* and it has been a common practice in laboratories to press out portions of these muscles between glass slides and examine them under the microscope. But these muscles are quite free of fatty tissue and when the same principle is applied to sausage the vision is obscured by fat droplets and various artifacts produced by the fat. It is almost impossible to find a piece of muscular tissue in sausage sufficiently free of fat to avoid this difficulty. Furthermore they are of various shapes and thicknesses thus increasing the difficulty in pressing out into a suitable layer for observation under the scope.

The following technic will eliminate many of the difficulties encountered.

Using a sharp knife or razor blade cut off the outer dried end of the sausage and select with a pair of fine pointed forceps a small piece of muscular tissue. Transfer the tissue for 1-2 minutes, to a small quantity of xylol poured into a watch-glass or other suitable vessel. Remove to a cover slip which has been laid out on a piece of glazed, white marble. Some of the xylol may be removed from the specimen by shaking. Cover immediately with a small drop of immersion oil and invert a slide over it in such a way as to prevent the formation of air bubbles. Place the two thumbs directly over the tissue and use sufficient pressure to flatten it out into a thin layer. The optimum thickness for observing under the scope is best learned by trial and error. There is most likely to be difficulty in leaving the specimens too thick. Invert the slide and cover slip again and examine under the low power of the microscope with the light reduced to a point where the structure of the muscle fibres is most plainly visible. There should be little difficulty in finding the coiled embryos in their lemon shaped cysts.

The principal advantage of this method is found in the large number of examinations which can be made in a short time. The importance of this factor is quite obvious in the case of sausage. A series of a dozen cover slips and slides may be laid out, one of the six inch pieces of sausage cut into 4-6 sections and 2-3 small pieces of muscular tissue selected from each for examination. The preparation of this number of mounts should require only a few minutes and the examination may be made at leisure. It is apparent that a rather thorough sampling of the sausage is possible under these conditions.

In order to have best success with this method the following points must be borne in mind :

1. The fat must be thoroughly removed with xylol or it will gather in droplets and layers diffracting the light rays and reducing visibility.

2. After removal from the xylol the tissue must not be allowed to dry before adding the immersion oil or it will be found impregnated with air which gathers in bubbles producing an effect similar to that produced by fat.

3. A sufficient quantity of immersion oil must be added, the amount required depending on the thickness of the specimen, or it is likely that air will be incorporated in the oil while pressure is being used to flatten the tissue.

4. Best results are obtained only when comparatively fresh sausage is available. After the lean tissue has become dry and hardened it does not readily flatten under pressure. Fairly good results have been secured, however, even with dried material.

5. It will be found that the cysts and larvae are more plainly visible immediately after preparation than after standing for 24 hours. This is probably due to gradual impregnation of the cyst with the immersion oil thus giving a uniform refractive index and allowing the light to pass directly through the larvae without being deflected.

The larvae may be made to stand out more plainly by tinting with a dye such as dilute basic fuchsin. This may be accomplished by pressing out the tissue between a slide and cover slip as described above but without adding immersion oil, covering it with xylol for 1-2 minutes, and washing off the xylol with alcohol. The alcohol is washed off with a dilute solution of basic fuchsin which is left on for 5-10 seconds. The stain is removed by dropping distilled water on it from an eye dropper. Blot carefully, add immersion oil and examine as usual. It has been found that less difficulty with air bubbles is encountered if the slide is warmed by passing through the flame and a large drop of immersion oil placed against one side of the tissue. After standing for 3-5 minutes the oil will gradually spread through the tissue almost completely eliminating the air. A little more oil may then be added and the slide carefully inverted over the tissue. The staining solutions are quite convenient for use when kept in dropping bottles so that a drop or two may be added to the specimen at will.

The principal difficulty in getting a good stained mount is due to an apparent thickening of the section in the staining process and

there is difficulty in knowing just how long to leave the stain on in order to get best results. A thick section cannot be stained as heavily as a thin section without obstructing its visibility under the scope.

The stained preparation requires much more time to make than the unstained preparation and is not necessary for detection of the trichina in fresh specimens. It is most valuable in preparing slides for permanent mounts.

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