

A New Technique for Counting Nematode Eggs in Sheep Faeces.

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Summary.

Particulars are given of a method whereby this work is simplified and the time required for each sample is reduced by about one-half (from four minutes to two minutes). The method involves the use of a specially prepared slide which is fully described. Such slides can be prepared in the laboratory and may be used repeatedly if simple precautions are exercised in washing them.

1. Introduction.

The method to be described and the special counting chamber slides employed were devised by the junior author (H.W.) who, as a laboratory assistant, has been carrying out routine egg counts during the past three years.

The method formerly used in this laboratory for enumeration of nematode eggs in sheep faeces was a modification of that devised by Stoll‡. Briefly, the technique was as follows:—

Two grams of faeces, weighed by crumbling into the balance pan, were placed in a glass jar of about 70 ml. capacity; 60 ml. water were added, and the mixture was allowed to stand for a period of 1 to 12 hours (in an ice chest at 4°C. if longer than 4 to 5 hours). To prepare for counting, the mixture was shaken and transferred to a $\frac{1}{4}$ -pint (about 150 ml.) glass cream jar, steel ball bearings (about 50 of 6 mm. diameter) were added, and the jar was corked and thoroughly shaken. A sample of 0.15 ml. was withdrawn with a pipette, placed on a slide, and covered with a $\frac{1}{8}$ -in. cover slip. By using a mechanical stage, it was ensured that all eggs in the samples were counted. The number of eggs counted, multiplied by 200, represented the number of eggs per gramme of the original faecal sample.

In most cases, duplicate samples could not be dealt with through lack of time, but since daily counts were usually made from the same individual sheep, slight errors from day to day would not be of great consequence.

The disadvantages of this method were:—

(a) *Time*.—After long practice (several months) one could complete the process, from mixing the faecal suspension to counting and recording the number of eggs, in about 4 minutes for each sample. It was often necessary to carry out as many as 50 egg counts daily in addition to other routine practices, so that actual mixing and counting occupied about 3 to 4 hours.

(b) *Debris obscuring eggs*.—Sheep faeces often contain large amounts of debris which renders observation of eggs difficult, unless one continually changes the focus and intensity of lighting.

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‡ Stoll, N. R.—“On Methods of Counting Nematode Ova in Sheep Dung.” *Parasitology*, 22: 116-136, 1930.

2. The New Technique.

The new technique is a combination of sampling and flotation methods. There were numerous modifications before the present method was adopted for routine use.

The technique is as follows:—

Weigh 2 g. of faeces by crumbling into the balance pan, place in a glass jar of about 70 ml. capacity, and add 30 ml. water. This mixture is allowed to stand for varying periods, usually not less than one hour. The longer the mixture stands, the easier is the breaking down of the faeces to a uniform suspension. Actually, counting may proceed after a very short interval—a few minutes—provided sufficient shaking with steel ball bearings is carried out to make a uniform suspension. When ready to proceed with counting, pour the faecal suspension into a suitable thick glass vessel—a cream jar ($\frac{1}{4}$ -pint) holding about 150 ml. is generally used. Rinse the glass jar with 30 ml. of the flotation fluid (formerly Sheather's sugar solution was used, but latterly saturated sodium chloride solution, which is cheaper and less "messy" to work with) and pour the rinsings into the cream jar. Add steel ball bearings, cork, and shake thoroughly. A sample is then withdrawn by means of a glass tube with a bore of 8 mm. diameter, with a rubber teat for suction, and allowed to run into a counting chamber on a specially made slide (see Fig. 1). Each chamber will contain about 0.2 ml. of the faecal suspension and has a ruled area which bounds a volume of 0.15 ml. The glass tube is used simply to withdraw the sample and not to measure it. The eggs rise rapidly and come to lie against the under surface of the glass slide which forms the top of the counting chamber. On this under surface is ruled the area which bounds the volume of 0.15 ml. On examination with the microscope, it is found that the eggs are all in the same focus against the slide, while the debris is out of focus on the floor of the chamber.

The size of the area to be traversed in counting the eggs varies according to the depth of the counting chamber. It has been found convenient to have an area of 1 sq. cm., which allows a depth of 1.5 mm. In making the slides, the glass cross pieces (Fig. 1 (3)) were selected by measurement to fulfil these requirements. If the depth is too great (and in consequence the defined area small), the debris interferes with the lighting and renders it difficult to see the eggs without changing the focus and light intensity.

Counting is a rapid process, owing to the relatively small area to be traversed and the absence of the necessity for continual focussing to see the eggs clearly. Not only has the speed with which the egg counting procedure can be carried out been considerably increased, but the whole process has been rendered easier and less tedious. It is now possible to carry out 50 egg counts in about 100 minutes.

The accuracy of this method has been checked against the modified Stoll technique used here formerly, and a close agreement has been found.

There is one disadvantage which is sometimes serious. If there is much dark-coloured faecal debris, it tends to interfere with light coming through the counting chamber and makes it difficult to see eggs

clearly without some change of focus and alteration of light intensity. This trouble has been overcome by using a piece of wire gauze (12 meshes per cm.) across the 8 mm. orifice of the glass tubing used for taking up the samples to be counted. The faecal suspension is drawn in and forced out through the gauze several times before a sample is transferred to the counting chamber. Check tests have indicated that no loss of eggs occurs, in spite of much faecal debris being excluded.

The presence of numerous small air bubbles in the preparation is also a definite disadvantage because the bubbles are in the same focus as the eggs and tend to confuse counting. Practice with the technique, however, especially when introducing the faecal suspension into the counting chamber, enables one to reduce the bubbles to such an extent that they do not interfere with counting. It has been found also that, if the preparations are allowed to stand for about an hour, the majority of the air bubbles disappear.

The special slide.

This is shown in Fig. 1, which is more or less self-explanatory. The glass cross pieces, which support the upper slide bearing the ruled areas, are cut from measured slides. Slides of even thickness are selected and according to this dimension the size of the ruled squares is calculated. The slides at present in use were made by the junior author (H.W.), Canada balsam being used to attach the various sections. In the present routine work (involving 50 to 100 egg counts daily) about 30 slides, each with three counting chambers, are in use. In actual practice, when counts are being carried out daily on the same individual sheep, duplicate counts are not made. It is a simple matter, however, to examine each faecal sample in triplicate, and to use the mean result.

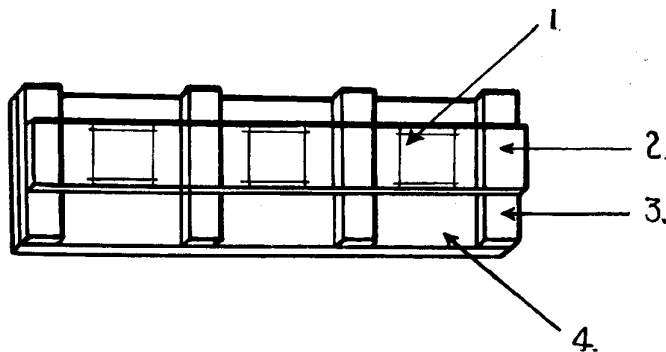


FIG. 1.—1. Ruled area of 1 sq. cm. on under surface of 2. 2. Cover slide
3. Supporting strip 1.5 mm. in thickness. 4. Basal slide.

It has recently been found that satisfactory counting chamber slides can be made of stout celluloid, using acetone to cement the pieces together.

Slides are cleaned in cold, running water immediately after use, shaken, and drained by standing on edge. This does not seem to injure the Canada balsam joints, even after constant use. Hot water must not be used, nor must the slides be dried by heat.