QUANTITATIVE DIAGNOSIS OF CHRONIC FASCIOLOSIS

1. Comparative Studies on Quantitative Faecal Examinations for Chronic *Fasciola hepatica* Infection in Sheep

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Introduction

Techniques for faecal examination, ranging from a simple smear to elaborate quantitative methods, have been used to diagnose chronic infection with *Fasciola* spp. The aim is to concentrate the eggs from the voluminous faeces, and has been successfully achieved by flotation (Vajda 1927), sedimentation (Benedek 1943) or by fractional sieving (Willmott and Pester 1952; Dorsman 1956). Many such methods with different procedures and with different flotation fluids have been described (Döbel 1963).

There is need for a relatively accurate quantitative method, which is simple and quick and gives reproducible egg counts for studies on the biology of the parasite and for the evaluation of anthelmintic efficiency in the field.

A simple quantitative flotation technique with potassium mercuriiodide was described by Whitlock (1950), and the sedimentation technique (Benedek 1943) was modified for quantitative diagnosis by Boray and Pearson (1960). The present paper reports the comparative recovery of *F. hepatica* eggs added to fluke-free sheep faeces by these two techniques.

Materials and Methods

Eggs were recovered from the gall bladder of sheep infected with mature *F. hepatica*. They were counted individually in a plastic slide under a stereomicroscope and then washed with water into a 50 ml jar. The required number of eggs was mixed mechanically with 3 g faeces from fluke-free sheep kept in pens on a standard diet, and the samples were prepared according to each method. In the flotation technique the sliding top apparatus was used, the amount of faeces was reduced from 4 g to 3 g and only 30 ml of water was added instead of 50 ml as described by Whitlock (1950); so the multiplication factor became 10 instead of 12.5. This modification was suggested by Whitlock (personal communication) and the technique has been routinely used.

The method described by Boray and Pearson (1960) was also modified using a different slide for easier detection of eggs, particularly if the amount of sediment was increased. Faecal samples were collected in jars of approximately 50 ml capacity; 3 g samples for sheep, 6 g for cattle or 1 g for small laboratory animals were weighed. The sample was covered with approximately 30 ml tap water. The samples were left in a refrigerator for periods up to a month prior to examination. The soaked faeces were then mixed with an electric stirrer. To avoid splashing and contamination, a rubber stopper was slipped loosely over the shaft. The material was then filtered through a bronze sieve of 32 meshes/cm into a conical urine glass. The sample bottle was washed with water through the sieve with a fine jet from a wash bottle. The contents of the urine glass were then allowed to sediment for 3 minutes. The supernatant was discarded by means of a water-driven Venturi suction pump. The sediment was then carefully washed into a 10 x 1.0 cm test tube and allowed to sediment for 3 minutes. The supernatant was again discarded by means of a water pump. The basic principle of the method was that the rate at which eggs of *F. hepatica* sink in water is about 100 mm per minute, faster than that of most unwanted debris of the faecal material. The short sedimentation time therefore was essential.

The sediment was stained with 2-3 drops of 1% methylene blue and, after the dye was evenly distributed, it was washed on to the glass counting slide, 12 x 9 x 0.3 cm marked in 100 rectangles with a diamond pencil or glass cutter. Perspex walls 0.6 cm high were cemented to the slide to avoid leakage and to permit dilution and mixing of the sediment.

The prepared slide was examined under a low power (15x or 25x magnification) stereomicroscope and the number of trematode eggs counted. The small quantity of faecal debris was coloured, against which the yellow *Fasciola* and clear paramphistomid eggs were visible.

In the case of large numbers of eggs (that is, 1,000 to 10,000 eggs per gram (epg)), the eggs in the horizontal and vertical middle rows were counted and multiplied by five.

Seventy-five samples of 3 g containing 30, 300 and 3,000 eggs each respectively were examined by the flotation method.

Seventy-five samples of 3 g each containing 30 and 300 eggs respectively, and 52 samples containing 3,000 eggs, were examined also by the sedimentation technique. In addition, 23 samples containing 30 eggs, 300 eggs and 3,000 eggs respectively were examined as above, but 3 drops of detergent ("Comprox") were added to the samples before they were processed.

After the examination of the samples, the number of eggs per gram of faeces was calculated.

Results

Table 1 shows that only 2 out of 75 samples with 10 epg were positive by the flotation method, but eggs were detected in all samples by sedimentation.

TABLE 1

Recovery of Eggs of Fasciola hepatica *in Faeces by Flotation and Sedimentation Techniques*

No. of Eggs per Gram	No. of Samples (3 g each)	No. of Positive Samples	No. of Eggs in Samples	No. of Eggs Recovered	% Recovery of Eggs	Calculated Eggs per Gram		
						Mean	Range	% of actual epg
Flotation								
10	75	20	2,250	23	1.02	3.1	0-20	31.0
100	75	75	22,500	228	1.01	30.4	10-100	30.4
1000	75	75	225,000	2,568	1.14	342.4	140-510	34.2
Sedimentation without Detergent								
10	75	75	2,250	571	25.38	2.5	0.7-5.3	25.4
100	75	75	22,500	6,658	29.59	29.6	6-44.4	29.6
1000	52	52	156,000	16,678	32.07	320.7	158.3-521.3	32.1
Sedimentation with Detergent								
10	23	23	690	275	39.85	4.0	1.7-6.7	39.8
100	23	23	6,900	2,925	42.39	42.4	26.7-55.3	42.4
1000	23	23	69,000	25,848	37.46	374.6	231-503.3	37.5

In the flotation technique only about 1% of the eggs was recovered compared with about one-third of the total number of eggs by sedimentation. More eggs (about 40%) were recovered by sedimentation if detergent were added, but the amount of sediment increased considerably and the examination was more difficult.

In positive samples, the average calculated epg was about one-third of the actual epg by both flotation and sedimentation.

Eggs were not recovered from 55 samples, and only a single egg was recovered from 17 samples containing 10 epg by flotation. Only 1 or 2 eggs were recovered by flotation from 32 samples (43%) containing as many as 100 epg. At both 10 and 100 epg the recovery from a large proportion of samples was appreciably below the average calculated epg. More uniform recovery was achieved from the samples containing 1000 epg.

With sedimentation the variation of recovery from sample to sample with 10 epg was large, but some eggs were recovered from all samples. In 84% of the samples with 100 epg and 77% of the samples with 1000 epg the recoveries deviated only ± 30% from the average.

Discussion

The results showed that a flotation (Whitlock 1950) and a sedimentation technique (Boray and Pearson 1960), which were selected for routine egg counts because of their speed and simplicity, may be used with reasonable accuracy if the faeces contain 1000 epg or more. However, in lighter infections it was shown that because of the dilution factor, the successful quantitative diagnosis with the flotation technique would often depend on the recovery of one or two eggs. In these cases, the use of the sedimentation technique is more accurate and sensitive. Cattle, particularly adults, usually have low egg counts because of their resistance to infection, and the sedimentation technique would be more reliable.

The sedimentation technique is simple; no chemicals are necessary, it can be modified for quick field diagnosis, and it is suitable for the quantitative diagnosis of both *Fasciola* spp and paramphistomid infections. It was also found that in the flotation technique distortion of eggs occurred due to the concentrated flotation fluid, and differentiation between *Fasciola* spp and paramphistomid eggs was difficult.

The addition of detergent to the samples increased the sensitivity of the sedimentation technique, but the increased amount of sediment hindered rapid egg counting and is regarded as undesirable.

The results showed that in both techniques the average calculated epg represented approximately one-third of the actual number of eggs in the faeces. It was also shown that about one-third of the eggs was recovered from cattle faeces containing 100 epg.

As only approximately one-third of the eggs added were recovered, the new multiplication

factor in the modified flotation technique should be 30 instead of 10. In the sedimentation technique, for sheep faeces the number of eggs recovered from each 3 g sample would represent the actual epg; for cattle samples, the number of eggs counted in each 6 g sample should be divided by 2.

Summary

The recovery of *Fasciola hepatica* eggs added to fluke-free sheep faeces was compared using a quantitative flotation (Whitlock 1950) and a quantitative sedimentation (Boray and Pearson 1960) technique.

Some modification of both techniques was described. It was found that about one-third of the actual number of eggs in the samples was recovered by both techniques.

It was concluded that the sedimentation technique is more suitable for quantitative diagnosis, particularly when detection of light infections is required.

References

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BOOK REVIEWS

MONOGRAPHS ON VIRAL DISEASES

L'Expansion Scientifique Française has produced a series of fine monographs with good illustrations and bibliography of a number of viral diseases of domestic animals.

They are:—

La Peste Porcine Africaine: Lucas, Haag et Larenaudie
La Peste Bovine: Jacotot et Mornet
L'Anémie Infectieuse des Equidés: Goret, Michel et Toma
La Maladie Aujeszky: Lautie
La Peste Equine: Mornet et Gilbert
and a series in three volumes by Joubert et Mackowiak,
Le Virus Aptheux
La Fièvre Aptheuse Spontanée
La Lutte Anti-Aptheuse

These monographs run from 120 to over 500 pages and appear to provide a full and factual account of the diseases and aspects of control of the disease concerned. They are written in French, but this should not deter those who are interested in viral diseases. The prices vary but are close to 40F, and the publications are available from L'Editeur, L'Expansion Scientifique Française, 15 Rue Saint-Benoît, Paris-6e.

H. McL. Gordon

PATHOLOGIE DE LA PRODUCTION DU LAIT

This booklet* covers most of the techniques now used in the diagnosis of animal brucellosis. It is set out in sections in logical sequence which enables easy reference. The aspects dealt with include the ante- and post-mortem sampling of animal tissues and products, the isolation and culture of *Brucella*, diagnostic tests on serum, whey, mucus, semen and milk, the preparation and standardisation of diagnostic reagents, and the identification of *Brucella* species and types.

Enough concise detail is given to make this a very useful guide to laboratory work on brucellosis.

The bibliography includes 8 references to books and general monographs and 38 authors' references.

S.J.A.

*Pathologie de la Production du Lait, II Methodes de diagnostic biologique des brucelloses animales. G. Renoux et R. Gaumont. Centre National de Coordination des Etudes et Recherches sur la Nutrition et L'Alimentation, pp. 51. (Extract from Annales de la Nutrition et de l'Alimentation, 20 (1). (In French). Quai Anatole-France, Paris-7e. 1966. (No price available).