RESEARCH NOTE

A NEW TECHNIC FOR COUNTING *SCHISTOSOMA JAPONICUM* EGGS IN PIG FECES

AL Willingham[1,2*], MV Johansen[2] and EH Barnes[1]

[1]Danish Center For Experimental Parasitology, Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Frederiksberg C, Denmark; [2]Danish Bilharziasis Laboratory, Jægersborg Allé 1 D, 2920 Charlottenlund, Denmark

Abstract. An improved laboratory method was developed for counting *Schistosoma japonicum* eggs in pig feces, which involves filtration, sedimentation and centrifugation, but avoids toxic chemicals. It is sensitive, allows easy differentiation from similar-sized and shaped protozoan cysts, and permits evaluation of egg viability both by direct viewing of eggs and miracidial hatching. It was found to be significantly better at recovering eggs than the modified Bell filtration technic. The sensitivity, specificity and practicality of this technic make it our method of choice for studies on porcine schistosomiasis japonica.

Detection of eggs in feces is the most decisive method for the diagnosis of intestinal schistosomiasis. In addition, quantitative and qualitative evaluations of excreted eggs are important criteria for studying the epidemiology of the disease and evaluating prevention and control strategies. Several coprological technics have been developed to study excretion of schistosome eggs, including direct smears (Kato and Miura, 1954; Katz, *et al*, 1972), filtration (Bell, 1963; Visser and Pitchford, 1972), sedimentation (Faust and Meleney, 1924; Faust and Ingalls, 1946) and concentration in various solutions (Hunter *et al*, 1948; Ritchie, 1948; Blagg *et al*, 1955; Knight *et al*, 1976). These technics often vary in their convenience and sensitivity depending on which host and schistosome species are being studied.

Schistosoma japonicum eggs are quite different from those of other schistosome species in that they are small (approximately 60 μm × 80 μm), oval and lack a prominent spine. In addition, they are covered with a dense fibrous matrix to which host tissues and cells adhere, often preventing observation of the shell surface (Nelson and Bayliss, 1946; Ford and Blankespoor, 1979). This makes recovery, identification, quantification and quantitative assessment of this species' eggs more difficult, leading many investigators to rely on miracidial hatching tests for estimating fecal egg excretion. Early studies on *S. japonicum* in definitive hosts primarily used modifications of the sedimentation method first developed by Faust and Meleney (1924) for coprological assessment. This technic permits the use of a large amount of feces for deter-mining egg numbers and assessing their viability but is regarded as too time-consuming for large-scale investigations. More recent studies have relied on concentration of eggs in formaldehyde solutions (Ritchie, 1948; Blagg *et al*, 1955; Knight *et al*, 1976). However, the toxicity of these solutions poses a serious health risk for personnel involved. Although these technics were developed to diagnose and evaluate human infections they have also been used for livestock and other animals in both laboratory and field investigations (Pesigan *et al*, 1958; Moloney and Webbe, 1983).

An appropriate method for counting schistosome eggs in pig feces must also allow for the presence of *Balantidium coli*, a common protozoan parasite of the pig's large intestine. Cysts of *B. coli* tend to be spherical or ovoid with a diameter of 40 - 60 μm (Zaman, 1978) and therefore similar in size and shape to *S. japonicum* eggs making a discrimination between the two difficult. Thus, requirements for studying porcine schistosomiasis japonica necessitate the use of a coprological technic which permits easy differentiation of *S. japonicum* eggs from *B. coli* cysts in addition to being sensitive and allowing evaluation of egg viability. Unfortunately, none of the available coprological methods were

Correspondence: Tel: +45 35282775; Fax: +45 35282774; E-mail: awi@kvl.dk

found to fulfill all these requirements.

Apart from the role domestic pigs play in the transmission of *S. japonicum* in China, the pig has recently been recognized as a potential host model for human schistosomiasis japonica (Willingham *et al*, 1994; Willingham and Hurst, 1996). Hence, in order to make population biological studies on *S. japonicum* in the pig, we devised a technic for evaluating *S. japonicum* eggs in pig feces which involves filtration, sedimentation and centrifugation, and includes some of the procedures used in the methods of Faust and Ingalls (1946) and Visser and Pitchford (1972). Saline (1.2%) is added to a 5g sample of feces taken from a homogenized specimen (approximately 50*g*) to make a 100 ml solution which is shaken mechanically in a plastic container for 10 minutes and then washed with saline from a spray pump through a series of 3 sieves of 400 μm, 100 μm and 45 μm, respectively. The residue left in the 45 μm sieve is washed into a 350 ml sedimentation flask and left twice in the dark, using saline, for 20 and 15 minutes, respectively, after which the final sediment is washed into a 10 ml conical tube, capped and centrifuged for 1 minute at 100*g*. The supernatant is removed and saline is then added to the sediment to make a 2.25 ml solution. This solution is thoroughly mixed and 0.15 ml transferred onto a standard microscope slide using a pipette, covered with a 24 × 60 mm glass cover slip and microscopically examined for eggs. Three counts are conducted for each fecal sample, the sum of which is equivalent to eggs per gram feces. The remaining solution (equivalent to 4g feces) can then be washed with water and a miracidial hatching test conducted.

We have found this technic very useful for our research purposes as it permits evaluation of a large fecal specimen, avoids the use of toxic chemicals and the samples can be kept refrigerated for several days before being examined. By using the method we have been able to detect even low numbers of eggs in the feces of pigs. Coverslips enhance the efficiency of the technic by preventing eggs from being hidden by excessive fecal debris. Differences between *S. japonicum* eggs and *B. coli* cysts are usually evident but when in doubt can be resolved by using a higher magnification. Direct viewing also permits observation of active miracidial flame cells thus giving an indication of egg viability. The additional feature of conducting a miracidial hatching test on the same fecal sample also adds to the technic's usefulness.

In order to test the Danish Center for Experimental Parasitology (DCEP) method for counting fecal egg excretion, we compared it to a modified version of the Bell filtration-staining technic (Bell, 1963) which was conducted using a sample equivalent to 1g taken from a 5g fecal solution. Nine stool specimens taken between 7-9 weeks post-infection from pigs infected with low doses of *S. japonicum* were analyzed by both methods with three replicates of each method conducted on each fecal sample. In two samples, all counts from both technics were zero. These were excluded from the analysis. In five of the remaining seven samples the mean eggs per gram (EPG) from the DCEP technic was higher than from the modified Bell technic (Fig 1). On average the DCEP technic recovered almost twice as many eggs. The egg counts were square root transformed and examined by analysis of variance, using pig and sampling week, nested within pig as block factors, and the effect of sample preparation technic was significant ($p = 0.006$). As a measure of the variability of the technics, the coefficient of variation (CV = standard deviation/mean) was calculated for the three replicates of each technic for every sample. In five of the seven samples the CV for DCEP technic was less than the CV for the modified Bell technic, in one sample they were equal and in the remaining case (sample 5) the CV could not be calculated for the modified Bell technic because no eggs were recovered.

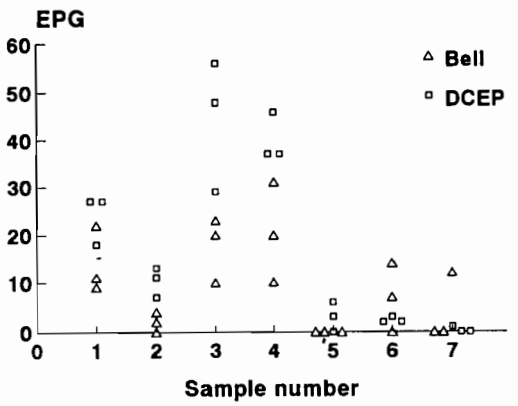

Fig 1–Comparison of *Schistosoma japonicum* eggs per gram (EPG) pig feces recovered using either the Danish Center for Experimental Parasitology (DCEP) method or modified Bell filtration method for counting fecal egg excretion. Three replicates of each method were conducted on each fecal sample.

The results of the methodological comparison indicate that the DCEP technic is significantly better at recovering eggs than the modified Bell technic and at least as good with respect to variability between replicates.

This work was financially supported by The Danish National Research Foundation. We are grateful to Lisbeth Pedersen and Rasmus Turin for technical assistance. Pigs used in this study were treated in accordance with the animal ethics laws of Denmark.

REFERENCES

Bell DR. A new method for counting *S. mansoni* eggs in faeces. With special reference to therapeutic trials. *Bull WHO* 1963; 29 : 525-30.

Blagg W, Schloegel EL, Mansour NS, Khalaf GI. A new concentration technique for the demonstration of protozoa and helminth eggs in feces. *Am J Trop Med Hyg* 1955; 4 : 23-8.

Faust EC, Ingalls JW. The diagnosis of schistosomiasis japonica III. Technics for the recovery of the eggs. *Am J Trop Med* 1946; 26 : 559-84.

Fause EC, Meleney HE. Studies on schistosomiasis japonica. *Am J Hyg* 1924; Monograph Series No. 3: 339 pp.

Ford JW, Blankespoor HD. Scanning electron microscopy of the eggs of three human schistosomes. *Int J Parasitol* 1979; 9 : 141-5.

Hunter GW III, Hodges EP, Jahnes WG, Diamong LS, Ingalls JW. Studies on schistosomiasis. II. Summary of further studies on methods of recovering eggs of *S. japonicum* from stools. *Bull US Army Med Dep* 1948; 8 : 128-31.

Kato T, Miura M. On the comparison of some stool examination methods. *Jpn J Parasitol* 1954; 3 : 35.

Katz N, Chaves A, Pellegrino J. A simple device quantitative stool thick-smear technique in schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo* 1972; 14 : 397-400.

Knight WB, Hiatt RA, Cline BL, Ritchie LS. A modification of the formol-ether concentration technique for increased sensitivity in detecting *Schistosoma mansoni* eggs. *Am J Trop Med Hyg* 1976; 25 : 818-23.

Moloney NA, Webbe G. The host-parasite relationship of *Schistosoma japonicum* in CBA mice. *Parasitology* 1983; 87 : 327-42.

Nelson ED, Bayliss M. Schistosomiasis japonica - laboratory diagnosis. *Bull US Army Med Dep* 1946; 5 : 673-80

Pesigan TP, Farooq M, Hairston NG, *et al.* Studies on *Schistosoma japonicum* infection in the Philippines. 1. General considerations and epidemiology. *Bull WHO* 1958; 18 : 345-55.

Ritchie LS. An ether sedimentation technique for routine stool examinations. *Bull US Army Med Dep* 1948; 8 : 326.

Visser P, Pitchford R. A simple apparatus for rapid recovery of helminth eggs from excreta with special reference to *Schistosoma mansoni*. *SAfr Med J* 1972; 46 : 1344-6.

Willingham AL, Hurst M. The pig as a unique host model for *Schistosoma japonicum* infection. *Parasitol Today* 1996; 12 : 132-4.

Willingham AL, Johansen MV, Vennervald BJ, Christensen NO, Nansen P. Experimental infection of Danish Landrace/Yorkshire crossbred pigs with *Schistosoma japonicum* from the People's Republic of China. *Acta Vet Scand* 1994; 35 : 395-400.

Zaman V. *Balantidium coli*. In: Kreier JP, ed. *Parasitic Protozoa*. New York: Academic Press 1978; 633-53.