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Authors: Whitlaw, Heather A., and Lankester, Murray W.

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A Practical Method for Cleaning Baermann Glassware

Heather A. Whitlaw and Murray W. Lankester, Department of Biology, Lakehead University, Thunder Bay, Ontario, P7B 5E1, Canada

ABSTRACT: When using the Baermann technique to detect larvae of *Parelaphostrongylus tenuis* in deer feces, it is difficult to ensure that no larvae remain on glassware between samples. Of several cleaning methods tested here, emersion in 95% ethanol after flushing with hot or cold water was the most effective and practical.

Key words: Baermann technique, cleaning glassware, *Parelaphostrongylus tenuis*.

The need to ensure clean glassware when conducting consecutive examinations of fecal material for nematode larvae using the Baermann technique (Beane and Hobbs, 1983) was summarized by McCollough and Pollard (1993). Both the funnels supporting the fecal sample, and the watchglasses into which larvae are drained, must be reliably cleaned and not retain larvae from one sample to the next. McCollough and Pollard (1993) recommended that Baermann glassware be sterilized by steam under pressure (autoclaved) prior to each use. However, this cleaning method is time consuming and is not feasible for institutions with limited equipment, nor in the field. Therefore, our objective was to develop an alternative practical method, suitable for use under a variety of conditions.

The testing procedure was divided into two components. The first involved direct observation of the response of approximately 200 first-stage, *Parelaphostrongylus tenuis* larvae pipetted in 0.2 ml of water into 5.5 cm diameter watchglasses and filled with 20 ml of various cleaning solutions including 95% ethanol, domestic bleach (5.25% sodium hypochlorite), and Sparkleen dish soap (Fisher Scientific Co., Pittsburgh, Pennsylvania, USA) mixed with hot tap water (53 to 55 C exiting the tap but cooling rapidly to 45 C in watchglasses). Also, larvae in a film of water covering the bottom of watchglasses (0.2 ml) were exposed to 750 watt, microwave radiation (Model 88922, 750 W, Kenmore, Sears Canada Inc., Toronto, Ontario).

Nematode larvae used in experiments were obtained from the feces of white-tailed deer (*Odocoileus virginianus*) on St. Joseph's Island, Ontario, Canada, in Lake Huron (45°17′N, 84°02′W), where *P. tenuis* has been found (E. M. Addison, pers. comm.). Fecal samples were stored at −20 C for 1 to 2 mo before use. Percent larval mortality after various exposure times in the watchglasses was determined by counting dead larvae, using a dissecting microscope at 16×. Larvae were judged to be dead when they were motionless, assumed the shape of the letter J or C, and did not move when stimulated gently with a pin. Standardization trials indicated that 4 to 6% of larvae recovered from frozen feces were already dead; up to 6% dead larvae was therefore considered zero treatment mortality.

We determined, in three replicate tests for each treatment, that 95% ethanol, undiluted bleach, bleach to water solutions of 1:1 and 1:2, caused 100% mortality of larvae in watchglasses in less than 5 min and all larvae were dead following microwave radiation for 3 min. Whereas, in soap and hot water, and a 25% solution of bleach, many larvae remained alive after 5 min (Table 1). Most larvae in 25% bleach after 5 min were tightly coiled and determination of death was difficult; by 15 min, all had uncoiled and clearly were dead. Accurate measurement of mortality rates using bleach solutions was further confounded when it was observed that 20 to 35% of larvae tested in unrinsed and lightly rinsed watchglasses that had previously contained 50% solutions of bleach, became inactive and appeared dead within 15 min. However, these same inactive larvae moved sluggishly when stimulated with a needle, and the overall mortality of these

TABLE 1. Mortality of *Parelaphostrongylus tenuis* larvae in watchglasses containing various cleaning solutions and following microwave treatment; each watchglass started with approximately 200 larvae.

Cleaning method	Treatment time	% mortality
95% ethanol	2 min	100
Hot water and soap	5 min	0
Bleach	30 sec	100
Bleach: water (1:1)	45 sec	100
Bleach: water (1:2)	3.5 min	100
Bleach: water (1:3)	5 min	20
Microwave radiation	15 sec	0
Microwave radiation	1.5 min	80
Microwave radiation	3 min	100

TABLE 2. Efficacy of cleaning Baermann funnels that previously contained 200 *P. tenuis* larvae each.

Number of positive funnels tested	Cleaning method	Number of cleaned funnels retaining live larvae	Number of live larvae recovered per funnel
95	Hot water, soap, and 95% ethanol	0	0
32	Hot water and 95% ethanol	0	0
15	Cold water and 95% ethanol	0	0
18	Hot water only	2	1 to 3
15	Cold water only	5	1
16	Microwave radiation for 1 min	1	1
16	Microwave radiation for 2 min	3	1 to 4

larvae after 12 hr in the dishes that probably retained trace amounts of bleach, did not differ from the mortality of larvae in dishes which had not previously contained bleach.

Secondly, we attempted to determine the efficiency of the various agents and treatments in cleaning glassware (glass funnels with 14.5 cm top diameter and 3.5 cm of neoprene tubing on the stem, and watchglasses) that previously had been used to examine fecal samples with greater than 15 larvae/g. Routinely, these positive funnels each contained approximately 30 g of deer feces suspended over porous tissue paper (Kimwipes, Kimberly-Clark Corp., Mississauga, Ontario) and, after 24 hours, 15 ml of water were drained into a watchglass and examined for larvae. Ninety-five sets of positive Baermann glassware were cleaned with soap and hot water, scrubbed with a sponge and bottle brush, vigorously rinsed in 95% ethanol for approximately 1 min, then refilled with water and allowed to stand 24 hr before being drained again into thoroughly cleaned watchglasses (soap, water, 95% alcohol). Approximately 5 l of ethanol were kept in a tightly sealed plastic bucket, into which funnels were dipped. Neoprene surgical gloves were worn while dipping the funnels. The same ethanol was reused to rinse several series of funnels. None of the funnels treated in this manner retained live or dead larvae (Table 2). In addition, none of the 32 funnels flushed only with hot water at moderate tap pressure and then rinsed in ethanol retained larvae, but two of 18 funnels flushed only with hot water did. In addition, one-third of the funnels flushed only with cold water (16 C) were contaminated (Table 2). Yet, of 15 funnels flushed with cold water followed with a 95% ethanol rinse, none retained larvae.

Conder and Williams (1983) initially showed that microwave radiation is effective in killing helminth and protozoan parasites in fecal material. We found that it also killed all *P. tenuis* larvae in a film of water on glassware if microwaved for at least 3 min. However, one of 16 funnels exposed for only 1 min to microwave radiation was contaminated with a live, first-stage larva (Table 2); dead larvae were found in a second sample. Of 16 funnels exposed to microwave radiation for 2 min, three retained live larvae (Table 2) and five others contained dead larvae. Interestingly, dead larvae only were recovered from funnels exposed to microwave radiation. Possibly, larvae killed by this method stuck to the glass and with re-filling, were loosened from the surface and later drained into watchglasses. In contrast, the rinsing action of the ethanol treatments

apparently removed all larvae from glassware. Another disadvantage of microwave radiation prevented us from increasing exposure times and ensuring the death of all larvae. Even after exposure of only 1 min, the rubber tubing on the funnels became scorched and hardened where it touched surfaces within the oven. Also, the inner surface of the rubber tubing became tacky and stuck together, preventing proper and easy drainage of the funnels.

The Baermann technique is widely used to detect dorsal-spined nematode larvae in feces. When searching for evidence of patent *P. tenuis* infections in hosts other than white-tailed deer, the numbers of larvae per sample are likely to be very low (Clark and Bowyer, 1986; Welch et al., 1991) and it is important that the funnels not retain any live or dead larvae from previous samples. A reliable cleaning method is especially important when consecutively examining fecal samples from white-tails and from other, alternate cervid hosts. McCollough and Pollard (1993) demonstrated that larvae will not be retained on autoclaved glassware. However, we have determined that, as a minimum, the same result can be obtained by merely flushing glassware with cold water followed by vigorous rinsing in 95% ethanol.

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