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Recirculating elutriator for extracting gastrointestinal nematode larvae from pasture herbage samples

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ABSTRACT

Gastrointestinal nematode (GIN) parasites present an important limitation to ruminant production worldwide. Methods for quantifying infective larvae of GIN on pastures are generally tedious, time-consuming, and require bulky equipment set-ups. This limitation to expedient data collection is a bottleneck in development of pasture management practices that might reduce pasture infectivity. We modified a soil elutriator concept for extracting GIN larvae from fresh herbage samples. Elutriators were constructed from readily available parts and compared to the Baermann funnel sedimentation method for larvae extraction. More samples could be extracted per day in the elutriator than in a Baermann unit with extraction times of 8 min versus 24 h, respectively. Accuracy, measured as maximum recovery of larvae seeded onto herbage samples, did not differ between extraction methods (62.3 vs. 69.8% for elutriator and Baermann, respectively, $P > 0.05$). Larvae recovery from herbage in elutriators showed a strong $\log_e$ relationship with extraction time ($r^2 > 0.98$), which will allow development of accurate correction factors for specific herbages to predict total larvae densities at extraction times less than those needed for maximum recovery. An extraction time of 8 min per sample gave the best compromise of speed, accuracy, and precision as measured by regression confidence bands and root mean square error of analysis of variance. Precision of the elutriator extraction for pasture samples was comparable to published methods and was not affected by forage species or canopy strata. The elutriator method was sensitive enough to detect differences in larvae density as small as 8 larvae g^{-1} DM among pasture treatments. Elutriators extracted nematode larvae from herbage samples with accuracy and precision similar to existing methods, but did it much faster. Elutriation shows promise as a rapid method for extracting infective GIN larvae from pasture herbage.

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1. Introduction

Gastrointestinal nematode (GIN) parasites are a primary limiting factor in small ruminant production worldwide (Waller, 2006). Development of management strategies to reduce the numbers of infective larvae on pastures requires understanding of the ecology of parasite species during their free-living stage. Infective larvae densities on herbage are a result of interactions among number and viability of eggs shed, environmental conditions that support egg hatch, and sward characteristics that affect migration into the grazed herbage horizon. Production losses occurred with ingestion of only 150 (Brown et al., 1985) to 400 (Steel et al., 1980) *Trichostrongylus* spp. larvae per day in Australia, and animal infection has been reported when forage contained no detectable larvae (Martin et al., 1990). This observation underscores the fact that quantification of

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GIN on pasture herbage is laborious, time-consuming, and prone to error both in sampling and in extraction of larvae from herbage. Simpler techniques may recover fewer larvae (Martin et al., 1990), but complex techniques require a greater investment of time and resources and may not offer enough improvement in accuracy to be worthwhile.

In general, extraction of GIN larvae from herbage samples has been approached via sedimentation or filtration. Variations of the Baermann sedimentation method are widely used for extraction of nematodes of all types from a variety of substrates. This method is based on the behavioral tendency of nematodes submerged in water to migrate downwards through filtering material and works best for nematodes that are vigorous and lively. Other sedimentation methods involve simply soaking herbage in a bucket of water. A wide range of sedimentation times from 6 (Levine et al., 1974) to 24 h (Couvillion, 1993) have been used. Additional time is required for sediment processing. The nematodes are collected and removed in tubing attached to the bottom of the funnels or buckets. Because plant debris and soil sediment along with larvae and can interfere with accurate counting, centrifugation and flotation steps are often added to clean the samples (Martin et al., 1990). Percentage recovery with this method tends to be low, usually less than 50% (Fine et al., 1993; Krecek and Maingi, 2004), although recovery up to 76.5% has been reported (Aumont et al., 1996). Recoveries are less when larvae density is low (Krecek and Maingi, 2004).

Filtration methods also rely on soaking forage samples in water, but often add agitation to speed transfer of larvae to aqueous suspension. Wash water is filtered through fine mesh screen or sieves to recover larvae. Filtration methods are generally faster than sedimentation because they do not rely on gravity or nematode migration, but there is risk of losing larvae through the screens or during transfer to counting media. Aumont et al. (1996) reported that filtering a pure clean suspension of larvae through a 20 μm screen recovered only 24% of larvae. The soaking required to dislodge larvae from herbage is the most time consuming step, with soak times of 3 (Eysker and Kooyman, 1993) to 24 (Aumont et al., 1996) h reported. As with sedimentation, centrifugation–flotation steps may be required to clean extracts before counting is possible. Maximum reported recovery of seeded GIN larvae using a filtration method is 40.8% (Krecek and Maingi, 2004), with other authors reporting ranges of 13–37% (Eysker and Kooyman, 1993; Aumont et al., 1996). In a direct comparison, Aumont et al. (1996) reported that a sedimentation method recovered 3.5 times more larvae than a filtration method and was more repeatable.

As can be seen from the preceding paragraphs, no method is 100% effective at extracting larvae from herbage. Correction factors specific to the method should be applied in order to obtain accurate densities (Krecek and Maingi, 2004). In addition to the time requirement, both sedimentation and filtration methods require large volumes of water and bulky extraction apparatus which further limits the number of extractions that can be performed per day. On top of the other challenges, fresh samples must be processed relatively quickly, because larvae counts begin to decline after three days of refrigerated storage (Fine et al., 1993). The difficulty of extracting necessary numbers of herbage samples in a timely manner has been a limitation to data collection that would enhance the understanding of GIN larvae ecology during the free-living stage.

In plant disease diagnostic laboratories, separation of plant–pathological nematodes in soil samples is frequently accomplished using a third method, elutriation (Byrd et al., 1976), which works on the principle of running air from bottom to top through aqueous suspension of sample, and differentially separating suspension components of varying densities. Heavy soil particles sink, large roots and organic particles are caught on the lip of the funnel, and fine particles and nematodes are flushed to the surface, spill over the edge and are caught in fine filters. This extraction method is repeatable and fast, with an extraction cycle typically taking 2.5–8 min (Byrd et al., 1976). Correction factors specific to the elutriator are used to adjust counts to 100% (Byrd et al., 1976). Raw elutriator extracts can be counted as is, or subjected to centrifugation–flotation first if needed for cleaning.

We could locate no data on use of elutriators for extraction of GIN larvae from herbage samples. The design used in plant pathology testing laboratories is unlikely to be feasible with herbage samples because floating herbage would interfere with water movement and plug the filters. The objective of this research was to design, build, and validate an elutriator suitable for herbage samples in order to increase the analysis speed for counting migratory GIN larvae on forage plants. The elutriator unit was evaluated for its ability to recover known and unknown numbers of larvae from water and fresh herbage samples, and results were compared to the Baermann funnel sedimentation method.

2. Materials and methods

2.1. Construction and operation of elutriator system

Two recirculating elutriator units were built (Figs. 1 and 2). Water tanks consisted of 19-L plastic buckets with water inlets, outlets, and drains constructed from PVC fittings and plastic tubing. Water was recirculated through the tank at 15 L min^{-1} using a utility pump (Model PCL-010, Little Giant Pump Company, Oklahoma City, OK). Tanks were filled using a hose valve near the bottom of the tanks and cleaned between samples by draining water through a valve on the tank bottom. During extractions, water recirculated through two outflow pipes located on opposite sides 14 cm from the top. Water from the outflows was recombined into a single hose which drained into a filter screen placed on the top of the tank. The filter screens were constructed from PVC rings fitted with nylon mesh with 11-μm pores (Part # CMN-0012-D, Nitex brand, Small Parts, Inc., Seattle, WA). Filtered water passed back into the tanks. Pressurized air (0.5 L min^{-1}) passed through a disc-shaped (9.5 cm diameter × 2.5 cm thick) aquarium air stone on the bottom of the tank, with bubbles ascending through the herbage sample held loosely 10 cm under water between two iron retaining screens with 1.6-cm^2 pores (57% of area) placed 10 cm above the bottom of the tank.

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