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Research Brief

Rapid method for recovery of strongylid third stage larvae of parasitic nematodes from small soil samples

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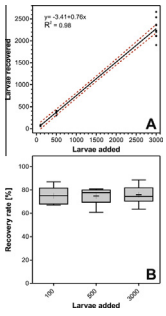
HIGHLIGHTS

- New method with mean recovery rate 75.3% for strongylid larvae from soil developed.
- Technique is suitable for soil samples from 50 up to 500 g.
- Low variability enables more precise estimation of number of larvae in soil.
- Easy, fast and affordable method.
- Examination of 20 soil samples up to 500 g each possible per day.

GRAPHICAL ABSTRACT



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ABSTRACT

Livestock with access to pasture is generally exposed to infections with parasitic nematode species by uptake of infective third stage larvae (L3) with the grass. L3 can survive on pasture and particularly also in the soil up to several months and sometimes even longer, depending on temperature and humidity. As indicators for health and productivity of grazing animals it is important to determine the intensity and species spectrum of parasitic nematode larvae by analysing grass as well as soil samples. A rapid method for the recovery of L3 using a centrifugal-flotation technique from soil samples of 50–500 g was developed. The method takes advantage of the low specific weight of larvae to separate them from equal sized soil and debris particles by centrifuging them in a saturated sugar solution. A stack of differently sized sieves is used to achieve elimination of larger particles, dust and sugar from the sample to enable easy counting of larvae. Independent of the number of larvae used for inoculation of the samples a mean recovery of 75.3% was obtained. The recovery rates obtained ranged between 60.8% and 88.0% which demonstrates a considerably lower variability compared to earlier approaches and therefore a more precise estimation of the actual numbers of parasite larvae in soil is achieved. Further advantages over already developed methods are the use of easy, affordable and eco-friendly materials, the simplicity of the procedure and a faster processing time with the possibility to examine up to 20 samples per day.

1. Introduction

Livestock kept on pasture is always exposed to infections with various parasitic nematode species, e.g. gastrointestinal strongyles, by uptake of infective third stage larvae (L3) with the grass (Nogareda et al., 2006). The L3 can survive on pasture and particularly also in the soil up to several months and sometimes even longer, depending on temperature and humidity (Boag and Thomas, 1985). The density and species spectrum of parasitic nematode larvae on pasture is an important factor for host health and productivity (Stromberg et al., 2012). Therefore it is of interest to obtain respective data by analysing grass as well as soil samples.

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Herein, a new and rapid method for a more accurate estimation of the strongylid third stage larvae (L3) content in small soil samples was developed and evaluated. For an accurate research on strongylid parasites precise, sensitive and reproducible recovery methods are necessary in order to estimate infection pressure on pasture. A number of different techniques for the recovery of L3 from herbage samples have been developed (Callinan and Westcott, 1986; Krecek and Maingi, 2004; Martin et al., 1990; Silangwa and Todd, 1964; Smeal and Hendy, 1972). However, it has been known for a long time that larvae move constantly on and off herbage (Crofton, 1948) and accordingly, different scientists have investigated the distribution of larvae on herbage and to a lesser extend also in soil.

The species of grass as well as the form of the leaves (Silangwa and Todd, 1964), composition of herbage mixtures (Callinan and Westcott, 1986) or the effects of temperature and humidity (Crofton, 1948) were found to alter larval occurrence on herbage. Surprisingly most parasitologists concentrated on the recovery of L3 from herbage and neglected the importance of the part of the larval population present in the soil. For instance, Williams and Bilkovich (1973) work regarding the distribution of L3 on pasture which was often cited (Forbes, 2008; Grønvold and Høgh-Schmidt, 1989; Hansen et al., 1989; Ward et al., 1991) stated that the number of larvae recovered from soil is so low that they do not contribute significantly to the overall population density. Accordingly, in publications regarding field experiments only herbage (but no soil) samples were investigated (Fiel et al., 2012; Gruner and Sauve, 1982; Nansen et al., 1988). Contradictory to this it was observed in previous experiments that a high proportion of the larval population on herbage migrates into soil. While methods for successful recovery of high proportion of parasitic nematode larvae from herbage has been developed (Demeler et al., 2012; Fine et al., 1993; Matthee et al., 2002; Sweeny et al., 2012), a precise, reproducible and standardised method for the recovery from soil is still missing in parasitology. Noteworthy, the recovery rates of nematode L3s from soil with previously published passive methods are very diverse with 55–94% recovery achieved by reverse water flow and sieving (Durie, 1959), and 42% (Callinan and Westcott, 1986) or 64–110% by application of a centrifugation-flotation technique (Young and Trajstman, 1980).

For the recovery of plant and soil nematodes agronomists developed several methods including reverse water flow and sieving (Verschoor and De Goede, 2000), centrifugal-flotation technique (CF) (Kung et al., 1990) or sugar-flotation-sieving (SFS) (Byrd and Nusbaum, 1966; Freckman et al., 1975), which are all classified as passive approaches. Additionally methods including the active migration of larvae have been proposed as beneficiary if applied after one of the above mentioned initial recovery methods. Different modifications of the original Baermann technique have been reported (Cobb, 1918; Liskova and Renco, 2007; Viglierchio and Schmitt, 1983), all based on active migration of larvae from samples, which usually follow one of the above mentioned initial recovery methods. Freckman et al. (1975) compared passive and active migration methods and concluded that the passive methods are superior as they are more representative of the community of nematodes in the soil and also allow recovery of non-active, cryptobiotic or even dead individuals. Moser and Frankenbach (2009) compared Cobb's modified decanting and sieving method (with subsequent active nematode migration) with the method published by the International Organisation for Standardisation (ISO), where nematodes migrate actively from the sample into a collection bowl and concluded that Cobb's method, with additional sieving steps followed by active migration of the nematodes, results in better recovery rates. Barker et al. (1969) were able to prove that different nematodes and their different seasonal activity in soil lead to varying recovery results for the three compared methods, the Baermann funnel method, the CF and the SFS. Different contents of sand, small stones and clay in the soil sample may also influence the recovery rate. Therefore it is necessary to evaluate which method is the most appropriate regarding the soil types and nematode species in order to obtain the best possible recovery rate (McSorley and Walter, 1991).

For an accurate ascertainment of the larval content in soil it is also important to achieve not only recovery rates as high as possible, but also a small variation of those recovery rates. For most published methods the actual recovery rate is unknown and percentages of recovered larvae are based mainly on assumptions. The aim of the study was the development of a rapid and efficient method with a known recovery rate and a small variation. A review by Hooper et al. (2005) describes three main flotation methods which were used as a basis for the development of the new method. Compared to other previously published CF or SFS methods, the method originally developed by W.R. Jenkins in 1964 (Hooper et al., 2005) produced the most promising results in preliminary experiments. Accordingly this method was modified in order to adapt it to the soil parameters used in the described experiment.

2. Materials and methods

2.1. Nematodes and soil source

Infective third stage larvae (L3) of an in-house laboratory *Cooperia oncophora* isolate were used for inoculation of soil samples. Larvae were obtained from experimentally infected calves by standard faecal cultures. All animal experiments were approved by the local authority (Landesamt für Gesundheit und Soziales, Berlin). All larvae were baermannised immediately prior to every experiment to ensure that only viable larvae were used. For inoculation of the samples three aliquots (each 10 µl) of larval suspension were counted and the suspension adjusted based on the mean larval counts and according to the desired total number of larvae (100, 500 and 3000 L3).

Potting soil of commercial quality was purchased in a local gardening shop and autoclaved under pressured steam at 134 °C for 20 min to eliminate bacteria, fungi or soil nematodes in order to exclude any interference on the recovery of the L3. In later experiments (inoculation with 500 L3) instead of autoclaving heat deactivation of the soil at 100 °C for 30 h was used.

2.2. Establishment of the method

On a calibrated laboratory scale 50 g of autoclaved soil were weighed out and transferred into a 400 ml glass with a plastic screw cap. Splashes of tap water were added to each sample to mimic natural humidity pattern. For the first set of experiments every sample was inoculated with either 100 ($n = 9$), 500 ($n = 6$) or 3000 ($n = 9$) *C. oncophora* L3, mixed and incubated overnight at room temperature so the larvae had enough time to creep deeper in the soil. The samples were examined by two researchers to determine the reliability and reproducibility of the procedure. For the recovery of L3 from the soil samples a subsequent sieving process was performed. For this purpose a stainless steel sieve with a pore size of 200 µm was placed on top of a 25 µm sieve and the stack of sieves were placed onto the rim of a plastic bucket. The soil sample was carefully washed through the sieves using a jet of tap water from a douche. Bigger particles (small pieces of wood, stones) remained on the 200 µm sieve, very small particles were flushed through both sieves into the bucket and were discarded. Soil-washing was stopped when no more small particles occurred in the jetted water in the bucket. Larvae and other particles