

Short Communication


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The number of *Oesophagostomum* spp. larvae recovered in coprocultures varies with media used

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Abstract

Nodular roundworms (*Oesophagostomum* spp.) are frequent parasites of the large intestine in several mammal species including humans and pigs, and their study often requires the use of infective larvae produced using several coproculture techniques. However, there is no published comparison of techniques to determine which yields the highest number of larvae. This study compares the number of larvae recovered from coprocultures made with charcoal, sawdust, vermiculite, and water in an experiment repeated twice using feces from a sow naturally infected with *Oesophagostomum* spp. at an organic farm. A higher number of larvae were recovered from coprocultures using sawdust relative to other types of media used, and this was consistent across the two trials. The use of sawdust to culture *Oesophagostomum* spp. larvae is rarely reported and our study suggests it can yield higher numbers relative to other media.

Introduction

Nodular worms (*Oesophagostomum* spp.) are frequent parasites of the large intestine in pigs, ruminants, primates, and rodents after hosts ingest infective third stage rhabditiform larvae that develop from first-stage larvae hatching from eggs shed in feces. Eleven species of this parasitic nematode have been described, some of which can be especially pathogenic in humans and domestic animals (Anderson 2000). It's common practice to culture parasite eggs in feces to harvest infective larvae useful in inoculums for experimental animals or for the identification of species. Coproculture of helminths is also useful for metabolic, immunological, genetic, developmental, and ultrastructural studies, as well as tissue culturing and evaluation of anthelmintics (Silverman & Hansen 1971). In coprocultures, feces from infected individuals are mixed with inert media to increase aeration and moisture for nematode parasite eggs to develop and hatch into larvae that molt twice before becoming infective. Several procedures used to culture infective *Oesophagostomum* spp. larvae from eggs in feces exist, but it's not known whether they yield similar number of larvae. For example, *Oesophagostomum* spp. third stage larvae have been harvested from pig feces mixed with vermiculite (Sellers *et al.*, 1975; Henriksen & Korsholm, 1983; Roepstorff *et al.*, 1987). Coprocultures of feces mixed with charcoal have been used to quantify *Oesophagostomum bifurcum* in human populations in Africa (Polderman *et al.*, 1991; Krepel *et al.*, 1995), and quantify the effects of high temperature on the infective larvae of *O. columbianum* (Premvati & Lal, 1961). Procedures originally developed for other nematode parasite species have also been used to culture *Oesophagostomum* spp. infective larvae. For example, *Oesophagostomum radiatum* larvae have been cultured by mixing peat moss with feces (Douvres, 1962), as was used for *Haemonchus contortus* (Cauthen, 1940) and *Hyostrongylus rubidus* (Kendall *et al.*, 1969). One other possible method for culturing larvae that has not been frequently explored in the study of *Oesophagostomum* spp. is mixing infected feces with sawdust as was done for culturing feces from sheep with mixed strongyle infections, including *Oesophagostomum* spp. (Agyei, 1995). In this study we compare the number of infective larvae recovered from coprocultures of an organic sow naturally infected with *Oesophagostomum* spp. prepared with charcoal, sawdust, or vermiculite to determine which method yielded more larvae.

Methods

An experiment comparing the number of larvae recovered using four different culture media was set up at room temperature (~22 °C) with vermiculite, coconut shell granular activated carbon charcoal (effective size 0.5 - 0.7 mm), medium grit chemical-free hardwood sawdust, and water as a control. The experiment was repeated twice using five replicate Petri dishes (145mm x 20mm)

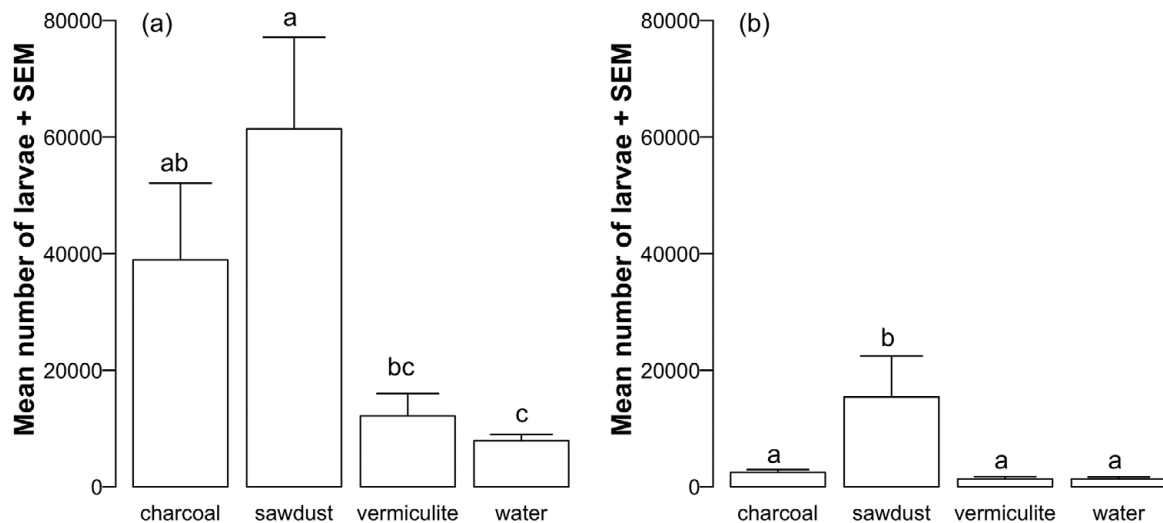


Figure 1. The mean number of *Oesophagostomum* spp. larvae (+SEM) cultured in feces mixed with charcoal, sawdust, vermiculite, or water (control) for 9 days at room temperature (22 °C) after (a) trial 1 (n= 5 Petri dishes per media) and (b) trial 2 (n= 10 Petri dishes per media). Bars with distinct letters indicate statistically significant pairwise comparison differences (Dunn's post hoc tests, $p < 0.05$).

per media type the first time, and 10 replicate dishes per media type the second time. Fresh feces were collected from a sow known to be naturally infected with *Oesophagostomum* spp. and managed organically at Rodale Institute (Kutztown, Pennsylvania, USA). Feces were brought to the lab on ice, and then for each replicate Petri dish, 15 g of feces was mixed with 15 g of culture media and 21 mL of tap water on Day 0 and subsequently sprayed with water every other day to remoisten the media as needed. Controls consisted of 3 g of feces evenly smeared on a 90 mm filter paper that was placed over the bottom half of an inverted 100 mm Petri dish placed inside a larger 145 mm dish that was then filled with water until the edges of the filter paper were immersed. On Day 9, larvae were harvested from each replicate dish using the Baermann technique (Baermann, 1917) and larvae from each dish stored in 20 mL of tap water in scintillation vials. The total number of larvae in each replicate was volumetrically estimated by mixing the contents of each vial with a magnetic stir bar and counting with replacement of the number of larvae under the 0.125 mL grid of three replicate McMaster slide chambers. The average of the three McMaster was then used to estimate the total number of larvae in 20 mL. Data were analyzed using R statistical software v.3.4.1 (R Core Team, 2017). A Shapiro-Wilk test on the number of larvae recovered showed data was significantly different from a normal distribution ($W = 0.59$, $p < 0.0001$). Therefore, statistical comparisons of the number of larvae recovered were done using non-parametric tests. A Wilcoxon rank sum test with continuity correction was used to assess differences in the number of larvae recovered between trial 1 and trial 2. A Kruskal-Wallis rank sum test was used to assess whether differences between culture media existed, and if they did, a Dunn (1964) Kruskal-Wallis multiple comparison procedure was run with p-values adjusted using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). The mean \pm SEM (standard error of the mean) for each culture media type are reported for trials 1 and 2. All statistical analyses used an alpha level of 0.05.

Results and Discussion

The number of *Oesophagostomum* spp. larvae recovered was higher in trial 1 than trial 2 ($W = 740.5$, $p < 0.0001$; Figure 1). As a result,

statistical comparison between the different media types was done separately for each trial. There were differences in the number of *Oesophagostomum* spp. larvae recovered between different media types in trial 1 ($KW = 13.753$, $df = 3$, $p = 0.003$), and in trial 2 ($KW = 19.677$, $df = 3$, $p = 0.0002$). In both trials, sawdust media produced a numerically higher number of larvae relative to the other three media types (Figure 1). However, in trial 1 only the sawdust vs. vermiculite ($Z = 2.62$, $p = 0.026$), charcoal vs. water ($Z = 2.51$, $p = 0.024$) and sawdust vs. water comparisons were different ($Z = 3.1$, $p = 0.011$), while in trial 2 the sawdust vs. charcoal ($Z = -2.33$, $p = 0.04$), sawdust vs. vermiculite ($Z = 3.93$, $p = 0.0005$) and the sawdust vs. water ($Z = 3.72$, $p = 0.0006$) comparisons were different (Figure 1).

Our results show that swine coprocultures using sawdust resulted in the production of a higher number of *Oesophagostomum* spp. larvae relative to the larva number produced using vermiculite or water controls. The number of larvae produced in charcoal coprocultures did not differ from sawdust in trial 1 but was significantly lower in trial 2 when a higher number of replicate dishes was used. Charcoal and vermiculite are the two most frequently cited media used in *Oesophagostomum* spp. coprocultures (e.g. Premvati & Lal, 1961; Sellers *et al.*, 1975; Henriksen & Korsholm, 1983; Roepstorff *et al.*, 1987; Polderman *et al.*, 1991; Krepel *et al.*, 1995). Yet, there are few published assessments of which technique leads to a higher yield of larvae for this nematode parasite. Studies comparing multiple culturing techniques have been done for nematodes infecting sheep (Agyei, 1995; Muchiut *et al.*, 2021), cattle (Berrie *et al.*, 1988; Steffan *et al.*, 1989), and goats (Nwosu *et al.*, 2006). However, a similar comparison of swine parasitic nematode coproculture techniques does not exist.

The higher number of *Oesophagostomum* spp. larvae recovered in this study using sawdust relative to vermiculite are unlike the results from sheep coprocultures which found no difference in the number strongyle larvae produced between sawdust and vermiculite media used (Agyei, 1995). Feces from calves experimentally infected with either *Ostertagia ostertagi* or *Cooperia oncophora* both yielded higher numbers of larvae in vermiculite and polystyrene pellet coprocultures when compared to coprocultures with no media added, and there were no differences between vermiculite

and polystyrene pellet coprocultures (Steffan *et al.*, 1989). In contrast, incubation of whole sheep fecal pellets instead of mixing with polystyrene pellets or vermiculite yielded more *Haemonchus contortus* larvae (Muchiut *et al.*, 2021). Vermiculite in this study produced *Oesophagostomum* spp. larva in numbers not significantly different than charcoal or water in both trials, but significantly less when compared to the number of larvae that sawdust yielded.

The purpose of mixing feces with inert media is to increase aeration and suitable moisture for nematode eggs to hatch and larvae to develop, thus improving larval yields. This study shows that coprocultures of swine infected with *Oesophagostomum* spp. yield higher number of larva when sawdust is used relative to other media such as charcoal or vermiculite. Although we did not measure the proportion of eggs that hatched, coprocultures were all mixed with the same fecal sample on the same day, making comparisons between media appropriate. The relative fewer number of larvae recovered in trial 2 relative to the first trial is likely related to natural variability in the number of *Oesophagostomum* spp. eggs shed by hosts over time (Roepstorff & Murrell, 1997).

Our comparison of coproculture media type used fecal material from a breeding sow singly infected with *Oesophagostomum* spp., yet concomitant infections in nature are the rule (Cox, 2001). It's therefore not known whether similar numbers of *Oesophagostomum* spp. larvae will be produced if sawdust is used in coprocultures mixed with feces from hosts with multiple parasite species infections. However, coprocultures using feces from cattle with mixed parasite infections have been shown to produce fewer larvae than when feces come from single infected host individuals (Berrie *et al.*, 1988). Nonetheless, our data support the idea that use of sawdust may be a preferable media to use in coprocultures to harvest larvae of *Oesophagostomum* spp. infective to pigs or humans.

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Conflict of interest. The authors declare none.

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