

# Comparative study of cultivation of feces in vermiculite or charcoal to obtain larvae of *Strongyloides venezuelensis*

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## ABSTRACT

**Introduction:** We compared feces culturing in charcoal or vermiculite to obtain *Strongyloides venezuelensis* larvae. **Methods:** Feces (5g) from infected rats was mixed with vermiculite (10g) or coal (10g) in plastic cups and incubated at 28°C for 48h. Larvae were recovered using Baermann-Moraes method. **Results:** Significantly higher number of positive larval cultures were recovered from vermiculite than from charcoal (15/17 and 4/17, respectively;  $p < 0.001$ ;  $990.6 \pm 307.5$  and  $215 \pm 78.1$  larvae,  $p = 0.027$ ). **Conclusions:** Vermiculite yields more larvae and provides cleaner pellets, improving larvae identification and facilitating their use for other purposes.

**Keywords:** Charcoal. *Strongyloides*. Vermiculite.

Cultivation of stool to obtain helminthic larvae is generally carried out with addition of different substrates or by spreading feces on plates containing solidified agar nutrients<sup>1,2</sup>. The most commonly used culture medium is coal, which must be pure, lacking additives, and, in particular, being free from colloidal silver. Moreover, visualization of the larvae or adult worms is rendered difficult due to the presence of coal particles. Agar culture is laborious and requires appropriate growth medium, adequate glassware, and sterilization systems.

Vermiculite is a compound formed by hydration of basaltic mineral; it has been used as a substrate for fecal culture, particularly in veterinary parasitology, as it has the advantages of being similar to soil and costing less<sup>3-7</sup>. It has also been used for growing insect<sup>8</sup> or nematodes larvae in order to evaluate the larvicidal effects of fungi<sup>9-12</sup>.

Very few reports have compared the culturing of stools in vermiculite vs. that with addition of other media. Steffan et al.<sup>13</sup> compared the addition of vermiculite or polystyrene pellets to cultivate cattle feces and found that both significantly improved growth of larvae of *Ostertagia ostertagi* and *Cooperia oncophora* as compared to the cultivation of feces without additives.

Agyei<sup>14</sup> compared the addition of vermiculite or sawdust to cultivate cattle feces to obtain L3 larvae of *Haemonchus*, *Trichostrongylus*, *Cooperia*, and *Oesophagostomum* and

concluded that the number of L3 larvae obtained from sawdust cultures was significantly higher than those obtained by other techniques; the former also did not require further elaborate apparatus.

As there have been no reports comparing the cultivation of feces in vermiculite and charcoal, we here compared vermiculite with charcoal as growth medium for larvae of *Strongyloides venezuelensis*. Feces of Wistar albino rats infected with *S. venezuelensis* (900 larvae, subcutaneously) were collected using an anticoprophagic cage. The eggs were quantified with a McMaster chamber. Two identical samples (in terms of weight and eggs per gram) of feces were homogenized with vermiculite (Vermiculite Insulation Thermo-Acoustic Ltd., Belo Horizonte, MG; granulation 1) or coal (Tobasa Bioindustrial, Tocantinópolis, TO; granulation 4) in disposable plastic cups (200ml) at a ratio of two parts of vermiculite or charcoal to one part stool (e.g., 5g of feces and 10g of vermiculite or charcoal). The cultures were incubated at 28°C for 48h, and were humidified daily with distilled water. The incubation time was based on the time used for cultivation of feces on coal for recovery of larvae of *Strongyloides stercoralis*<sup>2</sup>. Larvae were collected from the plastic cups using the Baermann-Moraes technique and counted in one aliquot of 50µL. The results of culturing in both media are summarized in **Table 1**. Of 17 cultures grown in vermiculite, 15 were positive, with recovery of filarial larvae and, exceptionally, free-living forms of the worm. In contrast, among the 17 cultures on coal, only four were positive. The two negative cultures in vermiculite were also negative in coal. When comparing the positive cultures, the mean number of larvae recovered was significantly higher in cultures containing vermiculite (**Table 1**).

In conclusion, data presented here demonstrated that fecal cultures in the presence of vermiculite, assessed

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**TABLE 1 - Comparison of feces culture in vermiculite or charcoal. Feces of rats infected with *Strongyloides venezuelensis* were mixed with vermiculite or charcoal and incubated at 28°C for 48h.**

Culture medium	Positive/total cultures (17)		P*	Larvae (n)** mean ± SD	P***
	n	%			
Vermiculite	15	88.2		990.6 ± 307.5	
Charcoal	4	23.5	<0.001	215 ± 78.1	0.027

\*Fisher's exact test; \*\*number of larvae in positive cultures; \*\*\*unpaired *t*-test with Welch's correction.

48h after cultivation, yielded significantly better results than cultures grown in the presence of coal, allowing recovery of a larger number of larvae. In addition, the pellet obtained by the Baermann-Moraes method is cleaner, greatly facilitating the identification of larvae and their evolutionary forms, and improving their application for other purposes, such as obtaining antigens and inoculation into experimental animals.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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