An improved method of using sawdust as a faecal culture medium for the production of 3rd stage strongylate nematode larvae

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SUMMARY

An improved method of faecal strongylate nematode (*Haemonchus, Trichostrongylus, Cooperia* and *Oesophagostomum*) ova culture, in which processed sawdust replaced animal faeces or peatmoss (vermiculite) as the medium is described. Sawdust was processed by sieving, washing and sterilized by autoclaving. Faecal strongylate nematode cultures were incubated in the dark at room temperature (24-26 °C), and relative humidity between 81 and 85 per cent, in wooden cupboards for 7 days. The yield of 3rd stage infective larvae (L_3s) in processed sawdust (171.1$\pm$ 3.61/g of faeces) was not significantly different (P >0.05) from that in vermiculite (181.0 $\pm$ 2.39 / g of faeces). Processed sawdust proved to be a better medium in producing L_3s than treated horse faeces in quality and cattle faeces both in quality and quantity. The L_3s produced from processed sawdust were highly active as observed from their rapid movements. The technique does not demand the use of elaborate facilities.

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RÉSUMÉ

Agyei, A. D.: *Une méthode améliorée utilisant la sciure de bois comme un médium de culture fécale pour la production de la 3e étape des larves de nématode strongilate.* Une méthode amélioreé de la culture ovulaire de nématode strongilate fécale (*Haemonchus, Trichostrongylus, Cooperia* et *Oesophagostomum*) dont la sciure de bois procédée a remplacé les excréments d'animal ou motte de tourbe (vermiculite) comme le médium, est décrit. La sciure de bois était procédée par le tamisage, le lavage et la stérilization d'autoclavage. Les cultures de nématode strongilate fécale étaient incubées dans l' obscurité à une température ambiante (24-26 °C), et une humidité relative entre 81 et 85 pour cent dans les placards en bois pendants 7 jours. Le rendement du 3e étape de larves infectives (L_3s) dans la sciure de bois procédée (171.1±3.61/g d'excréments) n'était pas considérablement différent (P>0.05) de celui de vermiculire (181.0±2.39/g d'excréments). La sciure de bois procédée ont donné la preuve d'être un meilleur médium en produisant L_3s que les excréments du cheval traités en qualité et les excréments du bétail en qualité et en quantité. Les L_3s produit de la sciure de bois procédée étaient profondément actives comme observé de leurs mouvements rapides. La méthode n'exige pas l'utilisation des facilités élaborées.

Introduction

Third stage infective larvae (L_3s) are important in carrying out controlled experiments in helminthological research apart from their usefulness in diagnosis. The need to culture or demand for L_3S in sufficient quantities and quality has led to the use of various materials such as animal charcoal, filter paper, sterilized animal faeces and peatmoss (vermiculite) as the culture medium to satisfy the need or demand (Baermann, 1917; Cauthen, 1940; Roberts & O'Sullivan, 1950; Douvres, 1960). However, unavailability of appropriate media or unsuitability of certain types of media makes it necessary to search for alternative sources. Horse faeces, for example, is not readily available in Ghana and cattle faeces has been found to be too compact (Roberts & O'Sullivan, 1950).

A preliminary study (CSIR, Ghana, 1986), in which raw, washed, and processed sawdust were compared, showed that sawdust collected from carpentry shops could be used as an improved medium for faecal culture. In this paper, after several trials in which peatmoss (PM), a standard material for faecal worm-egg culture in most labo-

ratories the world over, processed sawdust (PSD), horse (HF) and cattle (CF) faeces were compared, a method is described in which sawdust alone can be improved and used as a faecal culture medium.

Materials and methods

Processing of sawdust

Sawdust (SD) was collected from carpentry shops. The SD was taken only from timber or wood not previously treated with any chemical, and also excluded other extraneous materials. The SD was processed as follows:

a) SD was shaken through 1.5 - 2.0 mm mesh diameter sieve to obtain a homogenous material.
b) The sieved SD (about 1.3 kg) was soaked in hot boiling water (10 *l*), changing the boiling water until there was no more colour from the SD.
(c) Washed SD was oven-dried at 60 °C for 2 days, or dried in the sun to remove as much moisture as possible.
d) Dried SD, about 598 g was autoclaved at 15 1b / sq inch for 30 min. The product, now referred to as processed sawdust (PSD), was stored in polythene bags until needed.

Horse faeces (HF) and cattle faeces (CF) were dried and pulverized using a hammer mill. For sterilization, 842 g of HF and 1.6 kg of CF were autoclaved and stored as described for PSD.

Sources of worm eggs

Four strongylate-infected Djallonke sheep, consisting of three females, 2-3 years old and a male 3 years old, were confined in a pen to provide the worm-egg contaminated faeces. Additional strongylate-egg contaminated faeces were also obtained from animals in outside flock after estimating their worm-egg counts using the modified McMaster technique (Whitlock, 1948).

Preparation of faecal cultures

Kilner jars or enamel trays were used in the incubation of the cultutres. Worm-egg counts of all faecal samples were made using the modified McMaster technique (Whitlock, 1948) and therefore samples were pooled together and thoroughly emulsified. Total egg counts in the faeces from a flock was estimated by finding the mean egg per gram (epg) of all faecal samples taken from individual sheep in the flock. The mean worm egg count was then multiplied by the total weight of faeces collected from the flock.

Cultures were prepared by using the modified Baermann technique as described by Roberts & O'Sullivan (1950). The culture mixtures were made in proportions of one part faecal sample and two parts of culture medium (1:2). The PSD and the weighed faeces were mixed together thoroughly until a crumbly consistency was obtained; the amount of water added depended on the moisture content of the faeces. The Kilner jars or trays were filled with the mixture and covered with petri dishes or with glass sheets respectively. Cultures of horse faeces (HF) cattle faeces (CF) and peatmoss (PM) were made in similar manner. Cultures were examined and stirred periodically during incubation. Water was sprinkled on them when they appeared dry, and also to control fungal growth. All cultures were kept in a wooden cupboard at room temperature for 7 days.

Recovery of L_3s

The technique used was the modified Baermann technique as described by Roberts & O'Sullivan (1950). On the 8th day of incubation, Kilner jars were filled with water, covered with Petri dishes and inverted so that the jars stood in the Petri dishes (Fig. 1). About 15 ml of water was pipetted into the Petri dishes. The L_3s (Fig. 2) were recovered overnight, although for diagnostic purposes L_3s could be recovered after 2 h and examined. The L_3s from large cultures in trays were also recovered using the Baermann technique.

Yields of L_3 from cultures were collected and concentrated into 1 ml of water through centrifugation at 1500 g for 3 min. Total L_3 counts were made, after immobilizing larvae using Gram's iodine on microslides. They were identified by the possession of the 2nd larval sheeth (ADAS/MAFF, 1986), using a binocular microscope (Carl Zeiss Jena) at × 10 magnification.

Fig. 1. A Kilner jar inverted into a Petri dish, allowing the recovery of L_3s.

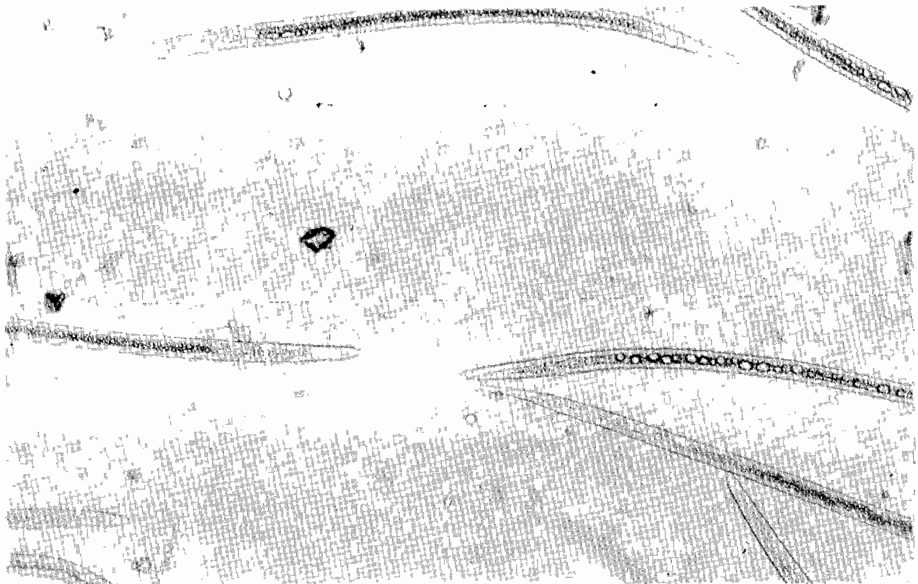

Fig. 2. L_3s of nematodes recovered from some cultures.

Survival and mobility of L_3s

The survival and mobility of L_3s were studied. Four test tubes each containing approximately 5000 L_3s were used. The four test tubes were divided into two groups, two test tubes in each group. The first was kept at 4 °C and the second

group at room temperature. Both groups were observed at weekly intervals for 3 months for mobility. The L_3s were considered dead after 30 min equilibration at room temperature.

Statistical analysis

The analysis of variance was used to determine statistical differences between the L_3 yields from the different culture media: PSD, HF and CF (Steel & Torrie, 1960). The Student-T test was used to test significance of mean differences between the number of L_3s from PSD and PM.

Results

Climatic conditions in the room

The temperature and relative humidity of the room was recorded daily at the same time throughout the period of the trial. The temperature ranged from 24 °C to 26.2 °C (mean 25.9 °C). The mean room relative humidity (RH) was 84.8 per cent and ranged from 81 to 95 per cent.

Recovery of L_3s

The L_3 yields were expressed as larvae per gram (L/g) of faeces obtained from the number of eggs per gram of faeces cultured. Significant differences ($P<0.05$) (see Table 1) occurred between PSD and HF on the one hand, and CF on the other hand ($P<0.01$). CF was found to give the lowest yield.

TABLE 1

Effect of Different Culture Media on Strongylate Nematode Larval Yields

Medium	*No. of cultures*	*Mean*
Processed sawdust (PSD)	36	171.1*
Sterilized horse faeces (HF)	24	184.5 NS
Sterilized cattle faeces (CF)	24	141.3**

* $P<0.05$
** $P<0.01$
NS Not significant

There was no significant difference between the yield of HF and PSD. Although larval yield was slightly higher in HF (Table 1), the L_3s from HF were qualitatively poor. Most of L_3s from HF were observed to be immature, as they were without the second larval sheath.

TABLE 2

Effect of PSD and PM on Strongylate Nematode L_3 Yields

Medium	*No. of cultures*	*Mean*
Processed sawdust (PSD) *t*-value	12	171.1
		0.32 NS
Peatmoss (PM) *t*-value	12	181.0

NS - Not significant

There was also no significant difference between larval yields from PSD and PM (Table 2).

Length of survival and mobility of L_3s

L_3s kept at 4 °C and at room temperature were very mobile for up to the 3rd week; however, those kept at room temperature were sluggish and a greater number were either immobile or dead after the 4th week. Those preserved at 4 °C were usually sluggish when initially brought out from the refrigerator, but soon became active when the temperature of the fluid reached room temperature. A few dead L_3s were observed after 12 weeks.

Discussion

The result of the present study indicate that PSD compared favourably with PM which is commonly used in most laboratories worldwide. Roberts & O'Sullivan (1950) did consider sawdust among several culture media studied to be suitable. However, they did not indicate whether or how the sawdust was treated. The removal of extraneous substances, through sieving and washing with hot boiling water and subsequent sterilization, in the present study, may have improved the SD. Though the mean ambient room temperature of 25.9 °C was below the 27 °C recommended for feacal culture in incubators (ADAS/MAFF, 1986), the ambient room temperature and room RH prevailing during the

study were enough to meet worm-egg hatching and larval growth requirements.

The quality of L_3 yields did not improve significantly with either HF or CF. The slightly greater count but poor quality of yield from HF cannot be explained. It has been indicated that increasing the incubation period from 10 to 20 days could improve larval yield from cultures incubated under room temperature (20 °C) conditions (ADAS/ MAFF, 1986). However, it has also be observed that increasing the incubation period from 7 to 10 days, under the prevailing ambient room temperature and RH conditions, did not improve larval yield in a preliminary study (Agyei, unpublished observations). Further, since the PSD yielded high quality L_3s, it was not considered necessary to increase the period of incubation. It should be stated that room temperature conditions in temperate areas most often range between 18 and 20 °C (ADAS/MAFF, 1986) which is considerably lower than the conditions under which this work was done. It was observed in this experiment that survival of the larvae was longer at 4 °C than at room temperature. This finding is at variance with that of Bucknor (1967) who found that L_3s, kept at room temperature probably under temperate conditions, survived longer. The short period of survival of those kept at room temperature might be due to their increased activity observed, in contrast to a much reduced activity at 4 °C. Increased activity *in vitro* is likely to have the effect of rapid exhaustion of food reserves for the L_3s.

Berrie *et al.* (1988) compared several larval recovery methods and found the recovery method used in the present experiment to be efficient in the recovery of L_3s. The L_3s obtained from PSD cultures were found to be highly active, and possessed all the characteristics of mature L_3s including the second sheath.

The present work has shown that SD, which is very easily available in Ghana, can be processed to provide a suitable culture medium,which yields highly active strongylate nematode L_3s. Further, as PSD is less messy and does not give strong offensive odours, its use is to be preferred to either HF or CF. The method described here does not demand any use of elaborate facilities.

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