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COMPARISM OF DIFFERENT METHODS OF ISOLATION OF PURE CULTURES OF *HAEMONCHUS CONTORTUS*

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ABSTRACT

This study investigated various techniques of producing pure cultures of the infective stages of Haemonchus contortus. Faecal samples (Group A) containing 1000 eggs per gram of faeces (epg) were routinely cultured in triplicates to develop infective stages while the adult worms were washed in normal saline and separated into two batches of 100 and 400 worms. The first batch (Group B) was incubated in normal saline under ambient laboratory conditions and laid eggs recovered by centrifugation. The second batch was crushed in a pestle and mortar to release the contained eggs and subsequently divided into four equal groups either directly cultured (Group C) or after washing by centrifugation in two changes of water (D), or the contained eggs floated out using saturated sodium chloride solution, washed and cultured (E) or washed, floated out and washed again before culture (F). In all cases, the eggs and crushed worm tissues were routinely cultured for 10 days to generate infective larvae. The percentage larval recovery from each method was recorded. Larval recovery was highest in the faecal culture group A (Mean = 879 ± 100 epg) which however contained infective stages of both H. contortus and other gastrointestinal strongyle worm species. This was respectively followed by group B (793 ± 864 epg), D (571 ± 495 epg), C (524 ± 485 epg), group F (291 ± 456 epg) and group E (179 ± 165 epg). These groups contained only infective stages of Haemonchus contortus. The results suggest that pure cultures of H. contortus infective larvae could easily be produced by either culturing eggs freely laid by individual adult females or crushed female tissues following washing with water to remove salt or other soluble worm tissue contaminants.

***Keywords*:** Isolation, Pure Cultures, *Haemonchus contortus*, infective larvae

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INTRODUCTION

Parasitic gastroenteritis (PGE) is a complex of diseases of ruminants contributed to by several nematodes in which *Haemonchus, Trichostrongylus*, *Oesophagostomum* and *Gaigeria* species usually predominated in Nigeria [1,2,3,4]. Effective control of PGE depends on efficient diagnosis and the establishment of the causative parasites among others. Several methods including the traditional coprology for nematode ova

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and post mortem examination for worm stages are available for the diagnosis of PGE. Post mortem examinations can only detect infections when the damage had already been done by the parasites. Consequently, detection of infection by examination for adult worms may not suffice for ante mortem diagnosis of nematode infections.

On the other hand, the traditional coprology for nematode ova is the oldest, simplest and most widely used technique in cattle and small ruminants but it lacks adequate specificity since the eggs of most nematodes (Trichostrongylids) responsible for PGE are similar in morphology and thus difficult to distinguish from one another [1,5,6]. Specific diagnosis of PGE is therefore usually done by feacal culture and larval recovery and identification [1,5,7]. Even when the larvae are recovered from feacal cultures and identified, it is almost impossible to isolate them to generate pure cultures of a specific nematode.

Pure cultures of nematodes are required for various purposes including: to provide live larvae for teaching and research; to eliminate the need for repeated sampling of accessible fluctuating habitats in search for living specimens; to provide all stages of development for observation, demonstration and experiments; to allow for the evaluation of more standardized methods for easier corroboration and the characterization of morphological and ecological variations under controlled and reproducible conditions.

In this study, we evaluated various techniques for the isolation of pure cultures of *Haemonchus contortus*, the most important and predominant nematode species infecting small ruminants in Nigeria.

MATERIALS AND METHODS

Study Site and animals

The animals used in the study were trade goats, predominantly of the Kano brown breed. They were randomly selected from the Nsukka slaughter slab in Enugu State located in the derived savanna zone of southeastern Nigeria. The animals included a total of 60 adult goats aged 1 – 6 years old [8,9]

Collection and Processing of Samples

Faecal samples were collected per rectum and egg counts determined by the modified McMaster technique [11]. Faecal samples containing ≥ 1,000 nematode eggs were routinely cultured (Group A). Faecal culture and larval recovery were done using the Baermann's technique as modified by Fakae *et al.* [10].

Following slaughter and evisceration, both ends of the abomasum of each selected goat were separated by ligature, dissected out from the rest of the viscera and the abomasums examined for adult stages of *H. contortus* as described by Hansen and Perry [11]. The worms were individually picked out into a Petri dish containing 2 ml of normal saline and the total number in each abomasums was recorded.

A batch of 100 adult female worms were individually picked out into a beaker containing 2 ml of normal saline and incubated at ambient environmental conditions for as long as possible (usually 2 – 6 hours) during which they remained alive and freely laid some eggs. Thereafter, the adult worms were picked out while the eggs were recovered by sedimentation after two changes of tap water to remove the salt content. These eggs, designated Group B, were cultured and the larvae recovered as earlier described.

Another batch of 400 adult female worms was crushed in 8 ml of tap water using a pestle and mortar to release their eggs. The crushed worm materials were divided into four equal parts (Groups C – F) and further processed as follows:

Group C: The crushed worm material was cultured directly (CR/C).

Group D: The crushed worm material was first washed in two changes of tap water and contained eggs recovered by sedimentation using centrifugation before culture (CR/W/C).

Group E: The eggs in the crushed worm material were floated out using saturated solution of

sodium chloride, washed in two changes of tap water by centrifugation and then cultured (CR/F/W/C).

Group F: The crushed worm material was washed in two changes of tap water before the contained eggs were floated out using saturated solution of sodium chloride, washed again and then cultured (CR/W/F/W/C).

In all cases, faeces, worm eggs and crushed worm tissues were cultured as earlier described while nematode stages were identified using standard parasitological criteria [11,12].

STATISTICAL ANALYSIS OF DATA

The data obtained during this study were summarized as percentages and means ± standard deviations and differences between the means analyzed using the one way analysis of variance (ANOVA). Probabilities (*P*) of 0.05 or less were considered significant [13].

RESULTS

Larval recovery and identification revealed that samples in Group A had mixed infections that comprised of *Haemonchus contortus* (60 %), *Oesophagostomum columbianum* (25 %) and *Trichostrongylus* species (15 %) species. On the other hand, cultures of Groups B – F yielded only the infective stages of *H. contortus*.

The result showed that larval recovery from laid eggs and crushed adult female worms ranged from 80 – 100% depending on whether the laid eggs or crushed female worms were cultured (Table 1). When laid eggs were used as a standard with 100% larval recovery, the larval recovery was highest (72%) from crushed female worms that were washed prior to culture followed by those cultured directly after crushing (66%). In general, larval yield was relatively higher when females were allowed to lay their eggs or the crushed parts were either cultured directly or washed with water prior to culture than when such eggs were floated out with salt solution prior to culture.

Table 1: Comparison of various methods of processing pure cultures of *Haemonchus contortus* larvae

Groups	Culture materials	No. (%) of larva hatched	Larval recovery Mean ± S. D. (range)	% larval hatch*
A	Faeces	40 (100)	872.4 ± 443.3 (50 – 6,250)	Not Applicable
B	Laid eggs	39 (97.5)	793.2 ± 864[a] (50 – 3,500)	100
C	Worm tissues crushed/cultured (CR/C)	40 (100)	523.5 ± 484.9[a] (50 – 2,000)	66
D	Worm tissues crushed/washed/ cultured (CR/W/C)	36 (90)	571 ± 494.5[a] (25 – 1,700)	72
E	Worm tissues crushed/Floated/ washed/cultured (CR/F/W/C)	34 (85)	178.5 ± 164.6[b]	23
F	Worm tissues crushed/washed/ Floated/ washed/cultured (CR/W/F/W/C)	32 (80)	291.4 ± 456.1[b] (25 – 3,500)	37

*Laid eggs were used as standard (100% larval hatch); [ab]Figures with different superscripts between laid eggs and the various crushed worm processing methods are significantly different.

DISCUSSION

The results revealed that the method used for culturing *H. contortus* eggs influenced the percentage hatchability and recovery of larvae. It is known that the hatching of nematode eggs and the survival of infective larvae are controlled by environmental factors such as temperature and moisture [1,5]. In this study in which various methods were used to process *Haemonchus* eggs which were then subjected to similar culture conditions, the highest number of infective larvae was recovered by either allowing individual live adult female worms to lay their eggs freely into a medium or culturing crushed female tissues; the former generating relatively more larvae than the latter method.

The relatively lower yield of larvae from crushed female worm tissues compared to freely laid eggs may probably be due to the presence of mostly immature eggs in the crushed worm tissues which ordinarily would not hatch to larvae. Previous reports have shown that immature worm eggs do not usually hatch to generate infective larvae either in natural or artificial culture conditions [14]. However, among the crushed female worm tissues, the most larvae were recovered from eggs cultured only after washing such tissues with water irrespective of whether or not they were first floated out with salt. The lower yield of larvae in cultures of female tissues not washed prior to culture may probably be due to the direct effect of the worm tissues or their by-products of fermentation that may inhibit egg hatch or larval survival or both within the culture medium. In a similar manner, the lower yield of larvae from samples floated out with salt prior to culture may be due to the adverse effects of the saturated salt on the eggs.

These observations, therefore, highlight the merit in washing of worm tissues following crushing or salt floatation prior to culturing in order to remove biological and salt contaminants that may inhibit egg hatch or larval survival or both. The results, therefore, suggest that pure cultures of *H. contortus* infective larvae could easily be harvested by either culturing eggs freely laid by individual adult females or by culturing crushed female tissues directly or following washing with water to remove salt or other soluble worm tissue contaminants. Prospective researchers wishing to generate pure cultures of nematode species should, therefore, ensure that such contaminants are as much as possible reduced or eliminated.

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