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Evaluation of Serum Pepsinogen Concentration as a Diagnostic Aid in Haemonchosis of Goats

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ABSTRACT. *Parasitic infection caused by the abomasal nematode, Haemonchus contortus is widely recognized as one of the most important diseases responsible for morbidity and mortality among goats in tropical and subtropical countries. Conventional diagnostic procedures using faecal egg counts have found to be unreliable in the diagnosis haemonchosis, particularly in subclinical infection which is the most common form of the disease and one that causes the greatest economic loss through reduced weight gain. In the present study an alternative diagnostic approach has been made by examining whether serum pepsinogen concentration could be used as a diagnostic marker in caprine haemonchosis as used in cattle and sheep for other nematode species that infect the abomasum. A total of 209 blood samples and abomasa collected from goats killed at an abattoir were examined for the presence of Haemonchus worms and the serum separated from blood was assayed for pepsinogen. The results indicated an increasing trend in the serum pepsinogen concentration parallel to that of the Haemonchus worm burden in the abomasum (r^2=0.5983). Serum pepsinogen concentration in goats is a moderately sensitive marker of haemonchosis and could be used as an adjunct in the diagnosis of the disease.*

INTRODUCTION

Morbidity and mortality resulting from parasitic gastroenteritis has been recognized as a major constraint (Devendra and Barns, 1983) to the development of the goat industry in tropical and subtropical countries, where the largest population of goats exists (Smith and Sherman, 1994). Parasitic

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infections, particularly in meat goats could have serious effects as shown in the recent studies by Faizal *et al.* (1999) which is reported that infected animals could lose up to 40% of their body weight in just one rainy season. Studies in Sri Lanka have indicated that *Haemonchus contortus*, *Oesophagostomum* spp. and *Trichostrongylus* spp. to be the frequently encountered species affecting the goats, and that *Haemonchus contortus* as the most common gastrointestinal nematode parasite (Van Aken *et al.*, 1990; Paranagama *et al.*, 1997). Other studies have indicated *Haemonchus contortus* to be the most pathogenic nematode species affecting the goats (Dorny *et al.*, 1995). The infection results in severe anaemia, a protein-loosing enteropathy, severe weight loss and death.

Faecal egg counts together with faecal culture are the widely used laboratory techniques to diagnose gastrointestinal nematode infections in ruminants. In goat however, these procedures have been found to be unreliable in the diagnosis of haemonchosis. Studies in cattle and sheep have shown that serum pepsinogen coupled with faecal analysis provides a more accurate reflection of the parasitic infection, particularly in the presence of abomasal parasites (Meana *et al.*, 1991; Scott *et al.*, 1995). Nematodes such as *Trichostrongylus axei*, *Haemonchus contortus* and *Ostertagia* spp. are known to cause mucosal damage of the abomasum resulting in the leakage of pepsinogen into the blood and the measurement of this pro-enzyme has been found to be a useful marker of abomasal injury caused by these parasites. In a preliminary study, Fox *et al.* (1991) showed an increase in the serum pepsinogen concentration with experimental haemonchosis but no attempt has been made to evaluate this analyte as a diagnostic tool for the disease and this has been pursued in the present study using material collected from an abattoir.

MATERIALS AND METHODS

Animals

Samples were collected from a total of 209 adult local or crossbred goats of either sex brought from the dry zone of Sri Lanka for slaughter at the Colombo Municipal abattoir.

Collection of samples

Prior to slaughter, the animals were tagged for the purpose of identification. Jugular blood (5 ml) was collected immediately before slaughter, serum separated and stored at -20°C until assayed. The abomasa of the respective animals were collected at slaughter and the adult worms in the lumen were counted as described by Ritchie *et al.* (1966). Abomasal scrapings digested in pepsin/HCl were used to determine mucosal larvae.

Measurement of serum pepsinogen concentration

In this assay, serum pepsinogen is converted to pepsin in an acid medium and the proteolytic activity of the latter is assayed using bovine serum albumin as the substrate.

Assay procedure

The assay was performed as described by Paynter (1992). Briefly, two sets of 75 μl of glycine-buffered pepsinogen substrate (3.2% bovine serum albumin (BSA), 1% Glycine, pH 1.6) were incubated with 50 μl of serum in eppendorf tubes. Both sets of tubes were incubated at 37°C, one was left for 30 min while the other was left for 210 min. The reaction was stopped with 250 μl 10% perchloric acid. All samples were centrifuged for 10 min at 12,000 *g* and 10 μl of the supernatant was assayed for protein using the bicinchoninic acid method (Pierce and Warriner, Chester, UK). The optical density of the samples on the microtitre plate was read at 570 *nm* on a microplate reader (CLS 963, Cambridge Life Science plc., UK). The difference in protein concentration before and after incubation was estimated by comparison with a standard curve of tyrosine concentration and the results were expressed as international units (i.u.).

RESULTS

Haemonchus contortus was the most common nematode in the abomasa of 209 animals examined in this study. Based on the number of parasites in each abomasum they were assigned to groups and ranked in an ascending order (Table 1). The pepsinogen concentration of each of the animals within the group was measured and the mean serum pepsinogen concentration for the group was computed.

Table 1. Mean serum pepsinogen concentrations of goats and the levels of *Haemonchus* worm burden.

Number of *H. contortus*	Number of samples	Mean serum pepsinogen concentration (i.u.)
0 - 49	40	1.2
50 - 199	46	1.4
200 - 299	31	1.7
300 - 499	37	2.6
500 - 699	20	3
700 - 999	18	5
1000 - 1499	11	5.01
1500 - 3750	6	6

A low worm burden (0-300) of *H. contortus* was associated with a low mean serum pepsinogen concentration (1.2-1.7 i.u.) while the increase of these abomasal parasites was associated with an increase in the pepsinogen concentration (Table 1).

Regression analysis of abomasal worm counts and serum pepsinogen showed that there was a positive correlation between serum pepsinogen concentration and *Haemonchus* worm burden (r^2= 0.5983); (Figure 1).

DISCUSSION

In cattle and sheep, serum pepsinogen concentration is widely used as a tool for the diagnosis of parasitic infections of the abomasum caused by *Haemonchus contortus* and *Ostertagia* spp. (Vercruysse *et al.*, 1987; Williams *et al.*, 1987; Fox *et al.*, 1991; Berghen *et al.*, 1993). However, these studies were based on animals reared under experimental conditions. In the present study, the potential of using the serum pepsinogen concentration could be

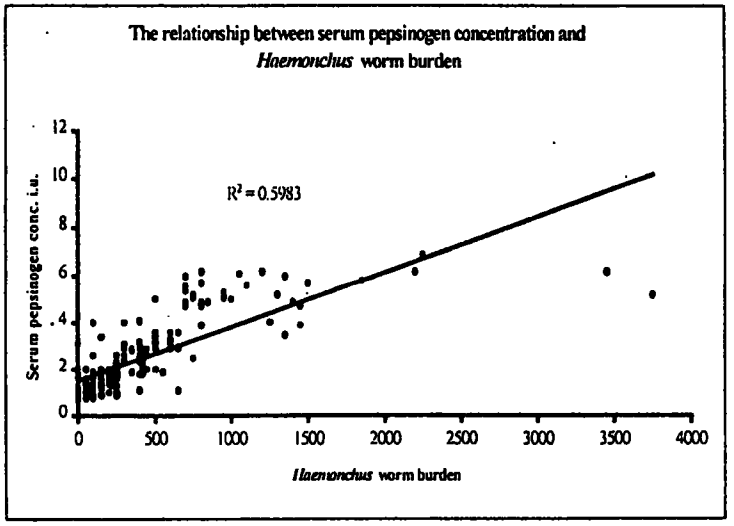

Figure 1. The relationship between serum pepsinogen concentration and *Haemonchus* worm burden.

used as a diagnostic tool for detecting haemonchosis was examined in goats reared under field conditions.

Fox *et al.* (1991) were the first to report an increase in pepsinogen with haemonchosis in goats but they did not explore its application in the diagnosis of the disease, instead they recommended gastrin measurements to be a more reliable marker of low *Haemonchus* infections. In the present study, an attempt has been made to use only the elevation of pepsinogen in haemonchosis as a diagnostic tool for the disease. The modest correlation of 0.5983 reported in this study reveals that serum pepsinogen concentration can be used as an adjunct procedure in the diagnosis of haemonchosis. However, it is also worth noting that other pathogenic nematodes such as *Trichostrongylus axei* can also cause tissue injury of the gastric mucosa resulting in an increase in serum pepsinogen concentration (Ford, 1976). Faecal culturing could be a way to overcome this confusion which may arise when interpreting the serum pepsinogen results. Indeed, other lesions of the abomasal mucosa such as erosions, ulcers and tumours can also elevate the serum pepsinogen but these lesions are rarely encountered among goats in veterinary practice (Smith and Sherman, 1994).

The correlation reported in this study was achieved using a large number of goats, therefore this test needs to be applied on a herd basis rather than on few animals or individual animal because the correlation may differ in the latter groups. In this respect the serum pepsinogen level has also to be used in addition to faecal egg counts where the validity of the latter procedure is more accurate in herds rather than in individual animals (Baker, 1988).

In the present study, many of the animals examined had a worm burden of less than 500, while a few animals had an infection exceeding 1000 worms. The low sample number in the high worm burden groups may have influenced the correlation coefficient reported herein. Unlike in an experimental infection, it is difficult to obtain many animals with high worm burden (above 1000) in a study of this nature since these parasites are likely to impose pathophysiological changes in the infected goats that threaten the very existence of such animals. Indeed, a study using experimental animals, which have very high worm burdens, is likely to produce a better correlation between the *Haemonchus* worm burden and the pepsinogen level in goats as reported for other domesticated ruminants. Moreover, a study of this nature will also help in better standardization of the assay and for establishment of appropriate cut-off values that will further extend the application of this assay as a field test.

CONCLUSIONS

The results of the present study indicates that the assay of serum pepsinogen level is a useful adjunct to faecal egg counts in the diagnosis of haemonchosis in herds of goats rather than in individual animals.

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