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## Variability in the Response of Yankasa Sheep to Graded Experimental Infections of *Haemonchus contortus*

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**Abstract:** The aim of this study was to evaluate the influence of different levels of *H. contortus* infections on parasitological and some haematological parameters in Nigerian Yankasa sheep as a basis for further genetic studies. Forty seven Yankasa rams were divided into 3 infected groups (n = 12) the fourth group (n = 11) served as control. The animals were infected with trickle infections of third stage larvae which totaled 35,000, 14,000 and 2800 for groups 1, 2 and 3, respectively. Faecal Egg Count (FEC), blood Packed Cell Volume (PCV), Peripheral Eosinophil (PE) response and Worm Burden (WB) at necropsy were used to assess resistance, over a 21 week period. The mean establishment rate decreased with increasing infection dose, being less than 2% of the infective larval doses, although there was wide individual variability in the response to infections irrespective of dose given. Thirty two percent of animals succumbed to infection in the first 4 weeks and these were regarded as susceptible animals and they had high FEC values (10,880-22,280), sharp decreases in PCV and low eosinophilic values compared to animals that survived to the end of the experiment. Surviving animals were refractory to the infections as shown by a delayed prepatent period (up to 49 days), low helminth egg output (626-1172 EPG) and low mean worm burden (44-357). There was negative correlation between FEC and PCV ( $r = -0.29$ ) which was significant ( $p < 0.05$ ) and also a negative but insignificant correlation between FEC and PE ( $r = -0.09$ ). These variable responses are indicative of acquired immunological responsiveness and resistance to infection with *H. contortus* which could be exploited to reduce production losses.

**Key words:** Sheep, *Haemonchus*, FEC, eosinophils, resistance

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### INTRODUCTION

Evaluation of sheep breeds in different breeding situations and environments have demonstrated the existence of substantial variations among sheep breeds in resistance to gastrointestinal parasites, with the possibility of breeding for nematode resistance (Gauly and Erhardt, 2001; Bishop and Stear, 2003; Baker *et al.*, 2003; Amarante *et al.*, 2004). This resistance has been shown to be genetically controlled and it is possible to evaluate the variation in resistance by using simple parameters such as faecal egg counts, PCV and circulating eosinophils which has been found to be repeatable, heritable and responsive to selection. Packed cell volume is particularly useful where the dominant nematode species is

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*Haemonchus* since, it sucks blood and blood loss has been found to be highly correlated with anaemia (Gauly and Erhardt, 2001). The relationship of traits of parasite infection such as FEC and haematocrit with intensity of infection has not been evaluated in the Yankassa sheep in Nigeria.

The Yankasa correspond with the Djallonké type sheep and are known for their adaptation to the tropical hot and humid environment of West Africa. They are the most widely distributed and most numerous breed in Nigeria and are usually kept under a semi intensive system in small units by peasant farmers in villages and urban areas. They serve as a ready source of cash and meat on festive occasions and the low initial investment required for these animals makes them attractive to small scale farmers even though productivity and profitability are low because of a number of factors which include parasitism and poor management (Fakae *et al.*, 2004).

Studies carried out with the West African Dwarf (WAD) goat of the subhumid zone of Nigeria have shown that they are endowed with strong resistance and resilience to *H. contortus* (Chiejina *et al.*, 2002, 2005; Fakae *et al.*, 2004; Behnke *et al.*, 2006). These studies identified two distinctive response phenotypes, namely strong and weak responders. These workers used the term haemonchotolerance to describe the resistance and resilience/tolerance of the Nigerian WAD goat to the nematode and considered it to be an attribute of the breed, with a possible genetic origin. Strategies to the genetic management of disease include choosing the appropriate breed for the production environment. Since, the Yankasa is more widely distributed in Nigeria, being found in all ecological zones, it may have acquired higher tolerance to haemonchosis, through several decades of natural selection in the different ecological zones. Therefore, this breed may be a more suitable one for selective breeding for resistance.

The aim and objectives of this study were to evaluate the response of Yankasa sheep experimentally infected with graded doses of *Haemonchus contortus* and which will be allowed to run its full course. Their response to various infection levels would help establish a baseline in the selection of individuals for breeding for resistance against *H. contortus* infection. This improved helminth control strategy will offer an opportunity to increase productivity and hence the livelihood of the resource-poor farmers who raise these animals.

## MATERIALS AND METHODS

### Study Area

The study was conducted at the Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, located at about latitude 11° 12' N and longitude 7° 37' N with an altitude of 610 m and annual rainfall of 1107 mm. The climate of the area is sub humid while the vegetative type is Northern Guinea Savannah.

### Experimental Animals

Forty-seven Yankasa rams with an average age of 12 months, were purchased locally and used for the experiment. All the animals were quarantined for 4 weeks and screened for internal and external parasites. Irrespective of infection or otherwise, all animals were treated with Panacur (Fenbendazole). Hoechst (AG) at a dose rate of 75 mg per 15 kg b.wt. Amprol (Amprolium. Merck sharp and Dohme) 35 mg kg<sup>-1</sup> as well as terramycin long acting (oxetetracycline. pfizer) 200 mg per 10 kg b.wt. All animals showed zero strongyle egg count at the beginning of the experiment.

Table 1: Infection protocol and number of sheep in the groups for the study of *H. contortus* larval infections

Groups	N*	Infection protocol	Total No. of larvae
1	12	5000 larvae per day×7 days	35,000
2	12	5000 larvae per day×7 days	14,000
3	12	5000 larvae per day×7 days	2,800
4	11	Nil	Nil

\* N: No. of animals in each group

The animals were maintained on concrete floor pens under conditions designed to prevent accidental helminth infections and zero grazed throughout the period of the experiment. They were fed hay *ad libitum* and a 12.7% protein concentrate ration. They also had access to water and trace mineralised salt blocks. No anthelmintic treatment was administered during the study.

### Experimental Design

Forty-seven animals were allotted into three groups of twelve animals each and eleven animals in the control group. Allocation of animals into groups was based on liveweight, balancing each group with the different weight range of animals. The sheep were infected with third larval stage (L3) of *H. contortus* according to the protocol shown in Table 1. Faecal and blood samples were collected from individual animals in the mornings. Faecal Egg Counts (FEC) were carried out daily between day 0 and day 14 and then weekly till week 5 and subsequently every other week up to 21 weeks post infection. Blood samples were collected weekly for 5 weeks and then every 2 weeks.

### Parasitological Procedures

Faecal egg counts were carried out using the McMaster method (MAFF, 1986). Infective L3 were obtained from cultures of faeces collected from monospecifically infected sheep. These cultures were maintained at 26°C for 10 days and the L3 recovered by the method of Hansen and Perry (1994). Larvae were stored at room temperature in petri dishes with water for a maximum of 2 weeks. Larval inocula for infection were prepared by counting the number of active sheathed larvae in six 50 µL aliquots of larval suspension. The larvae were administered orally, using a syringe on day 0.

### Haematology

Blood samples were collected into vacutainer tubes containing EDTA, by jugular vein puncture. Packed cell volume was determined by the microhaematocrit method (Hawksley and Sons, W. Essex, UK) and peripheral eosinophils by the method outlined by Dawkins *et al.* (1989).

### Worm Recovery/Count

All animals that died were necropsied and the worms present in them recovered and counted. The rest of the animals were humanely slaughtered at the end of the experiment. The abomasum was ligatured at both ends and removed. The abomasal content, washings and mucosal scraping were processed according to the method of Hansen and Perry (1994) and a 10% aliquot sample of each part was searched to recover all nematodes present.

### Statistical Analysis of Data

Values of FEC and worm counts were normalized by log<sub>10</sub> (EPG or worm count+1) transformation before statistical analysis. The data were analysed using the Statistical Package for Social Science (SPSS). Treatments were compared using analysis of variance, while the association between data was determined using Pearson's rank correlation.

## RESULTS

### Mean Establishment Rates and Worm Burden

The establishment rate was computed by dividing the number of established worms by the number of larvae given. The mean establishment rates were 1.01, 1.25 and 1.70% in groups 1, 2 and 3, respectively and these did not differ significantly ( $p>0.05$ ) in any group. No immature stages were found in the intestinal mucosa and no *H. contortus* were found in the control group. Mean worm burden and ranges are shown in Table 2. Worm burden in groups 1 and 2 did not differ significantly ( $p>0.05$ ) but worm burdens in both groups differed from that of group 3 at  $p<0.001$  and  $p<0.01$  significant levels, respectively. By the 21st week of infection when the experiment was terminated, the more heavily infected sheep retained more parasites than did the lesser infected sheep (Fig. 1) but the percentage survival of the worms relative to the infective dose was less.

### FEC and Clinical Signs

Faecal egg count for infected groups are summarised in Fig. 2 as arithmetic group mean values. The pre-patent periods of the infections fell between 14-49 days and were longer in the group 1 animals compared with the other two infected groups (Table 3). The early patency in groups 2 and 3 were mainly due to 1 animal each from these groups, along with a few other animals that became patent at week 5. These animals quickly reached peak FEC of between 10,880-22280 and died in the first four weeks of the experiment. With the death of susceptible animals, FEC in group 1, for example dropped by 92% by the 4th week and did

Table 2: Mean worm numbers and ranges of parasites recovered in sheep

Groups	Total No. of larva given per animal	Mean±SD	Range of parasites recovered per group
1	35,000	357.1±112.6 <sup>a</sup>	22-2205
2	14,000	175.3±54.1 <sup>a</sup>	30-750
3I	2,800	44.7±9.1 <sup>b</sup>	0-120
4	Nil	Nil	Nil

<sup>ab</sup>Means within columns with differing superscripts differ significantly

Table 3: Pre-patent period, peak and mean of faecal egg count of sheep given varying levels of *H. contortus* infections

Groups	Prepatent period	Peak FEC	Cumulative Mean FEC
1	22-49	1707 at week 7	626.0
2	14-22	2958 at week 4	982.2
3	14-22	3286 at week 7	1172.8

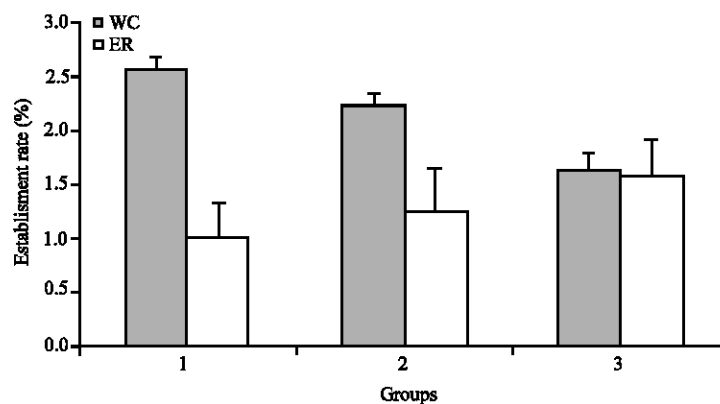


Fig. 1: Mean±SEM adult log transformed Worm Counts (WC) and calculated Establishment Rates (ER) of infection in sheep infected with varying levels of *H. contortus*

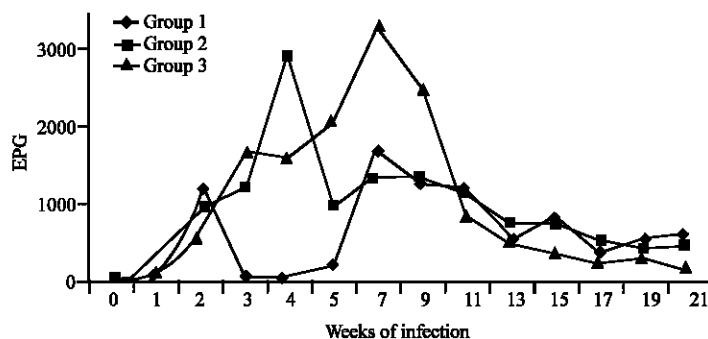


Fig. 2: Mean helminth Eggs Per Gram (EPG) of faeces in sheep infected with varying doses of *H. contortus*

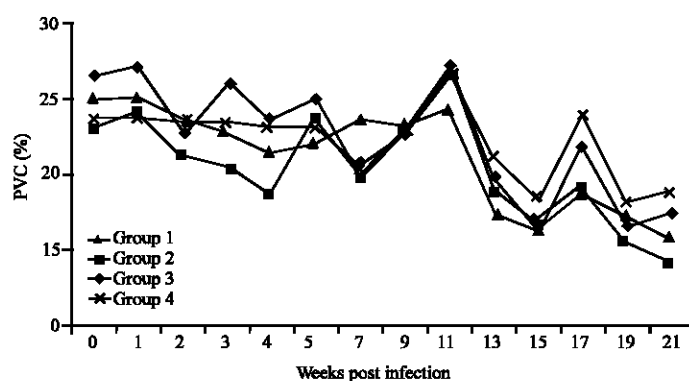


Fig. 3: Mean PCV values of *H. contortus* infected and control groups of sheep during the course of infection

not pick up again until the 7th week of infection. The peaks of weekly mean EPG occurred between weeks 4-7 in the different groups (Table 3). There was no significant difference between the overall mean FEC of all infected groups even though group 3 animals had the highest mean FEC, values being 626, 982 and 1172 EPG for groups 1-3, respectively.

There was a 32% mortality in the experimental animals in the first four weeks of infection. In group 1, seven animals died within the first 4 weeks of infection. In groups 2 and 3, respectively four animals each died during the same period. However, only one casualty was observed in the control group within this period. Clinical manifestations included lethargy, weakness leading to recumbency and prostration that terminated in death. At post mortem the abomasal mucosa showed petechia and oedema, with parasites on its surface and contents; fluid was also present in the pericardial cavities.

Cross section showed that there was hyperplastic thickening of the abomasal wall. Animals that died were mostly younger and lighter animals less than one year of age and weighed between 10-15 kg at the beginning of the experiment. No clinical sign of infection other than loss in body condition was observed in animals that survived to the end of 21 weeks. At post mortem however, their carcasses were pale and watery.

### PCV

PCV in all groups fluctuated with time and indicated that both infected and non-infected animals showed change over the course of the experiment (Fig. 3). Overall, PCV was

significantly lower in group 2, values being  $27.8 \pm 5.1$ ,  $25.5 \pm 5.1$ ,  $26.6 \pm 5.1$ ,  $27.4 \pm 4.0$  for groups 1-4, respectively). Haematocrit values showed an opposite trend to FEC during the 21 weeks of the study with a correlation coefficient of  $-0.29$  ( $p < 0.01$ ). Animals that died had critically low haematocrits in the range of 13-17%. While for surviving animals, PCV ranged from 19-32%.

**Peripheral Eosinophil (PE) Responses**

Infected groups showed rising eosinophil counts above the controls from week 2 of infection with peak eosinophilia counts occurring between weeks 5-13 (Fig. 4) in all infected groups. Overall, there was a negative but low correlation between PE and EPG ( $r = -0.09$ ) and this was not significant. Only group 2 had an overall change in eosinophil values that was significantly higher ( $p < 0.05$ ) than that of the control group values being  $4.3 \pm 2.4$ ;  $5.6 \pm 4.4$ ;  $4.4 \pm 4.5$  and  $3.9 \pm 2.4\%$ , respectively. Group 4 animals did not show any significant change all through the experiment, the highest value recorded for the group represented only a two fold increase over pre-infection values.

However, for infected groups, there was variation in individual and group animal responses that were obscured with the presentation of mean values. Therefore, the mean eosinophil values for 4 animals from each group that either succumbed or survived the infections are shown in Fig. 5 and 6, respectively. This is to provide an indication of how susceptible and resistant animals reacted to infection.

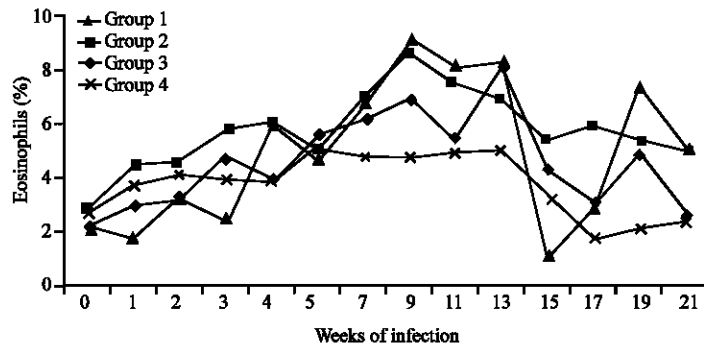


Fig. 4: Mean blood eosinophil values in the four experimental groups

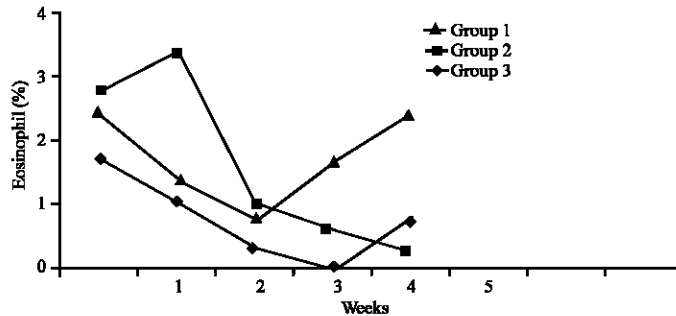


Fig. 5: Mean blood eosinophil values in animals in the infected groups (n = 4) that died in the first 4 weeks of infection

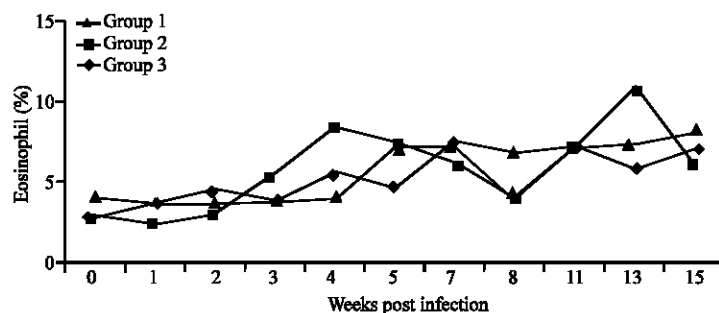


Fig. 6: Mean blood eosinophil values in animals in the infected groups (n = 4) that survived the course of the infections

## DISCUSSION

Resistance to nematodes is defined as the ability of animals to prevent the initial establishment of the infection and or reduce fecundity of worms that do establish or promote the elimination of worms that were able to colonize the gastrointestinal tract (Albers, 1987; Gaba *et al.*, 2006). Observations in this study indicate that the sheep were resistant to the doses of *Haemonchus contortus* larvae administered. Their response to the infections was considerably affected by the number of parasites administered, as this not only determined the time of patency, but also the number of worms that established, the rate of worm egg production and the rate of decline of egg production.

A longer prepatent period as was observed in group 1 animals that received the largest infection indicated that patency is affected by population density. This effect has been attributed to a crowding effect within the abomasum arising from competition for nutrients, optimal abomasal sites or to inhibitory metabolites produced by the larvae themselves (Le Jambre and Le Jambre, 1995; Gomez-Munoz *et al.*, 1999). It appears that this crowding effect also interferes with subsequent egg production as can be seen from Table 3 which shows that the higher the infection level, the lower the peak of egg production. This is in line with the observations of Gruner *et al.* (2003), who reported a density-dependent effect on fecundity in animals that are resistant. Faecal egg counts depend on both worm establishment and worm fecundity and both seemed to be affected in this study. This is shown by the fact that larvae administered serially for 7 days will be expected to produce a wave of increased FEC up to 9 weeks post infection as each batch of administered larvae come into patency. No such rise was observed, instead FEC pattern showed that there was great variability between individual sheep (0-22, 280) and the mean levels showing that worms in the lower infection levels were producing more eggs but their egg production quickly declined to low levels.

Younger and lighter animals succumbed to infection as was shown by mortality rates in this category. This conforms to reports on *H. contortus* in lambs that are less than 1 year old (Douch and Morum, 1993; Gamble and Zajack, 1992). Their greater susceptibility is thought to be a reflection of their poor capacity to manifest a specific immune response after infection, whereas in animals able to respond with specific antagonistic antibodies, the infection may largely be inhibited and quickly eliminated. This observation however contrasts with the findings of Rahman and Collins (1990), who used doses of 10,000-40,000 L3 in experimental primary and challenge infections in 2 to 5-month-old lambs and kids, respectively apparently without major pathological effects. These workers postulated that



resistance was not dependent on age per se but on other factors like weaning weight, nutrition or an immature haematopoietic system. In this study, resistance of older animals to infection seemed to be related to their ability to mount a response earlier and with greater intensity than younger animals and so limit establishment and egg production by infective larvae.

Observations in this study indicate that critical PCV values for survival of infections are above 17%. Anaemia is the main cause of production loss and death in infected sheep, the severity of anaemia in infected animals gave a good prognosis of infection. This is in line with the report of Fakaie *et al.* (1999) and Gruner *et al.* (2003), who related the ability of a host animal to resist infection with its ability to restrict blood loss caused by worms which do establish themselves.

The establishment rate of infection comprises the percentage of infective larvae that underwent evolution to adult stage and the number of worms that survive at necropsy (Gruner *et al.*, 2003). The number of worms that survive at necropsy has been described as a residual infection (Jarret and Urquart, 1971). The residual infection recovered from infected animals 21 weeks post infection was low (<2%) when compared to that of Agyei and Spong (1999) who got residual infection of 8.8% in WAD sheep 32 weeks post infection, in a derived savannah climate. This may be attributed either to the infectivity of the larvae used or that animals were able to throw off a greater part of the infections with time. The longer the interval between infection and necropsy, the greater the chances of worm expulsion, mediated by host responses or other factors.

The protective responses which result in the prevention of larval nematode establishment and reduced FEC depend on a cascade of events which include both humoral and cellular arms of the immune system and is under genetic control (Gill, 1991). Generally, T-lymphocytes, soluble cytoines, B-lymphocytes, plasma cells, various immunoglobulin isotypes, mast cells, eosinophils and globule leukocytes are known to actively take part in immunological reactions (Meeusen *et al.*, 2005; Pena *et al.*, 2006).

Eosinophilia has been reported to be correlated with protection against *H. contortus* (Terefe *et al.*, 2005) and so a potential marker for resistant individuals (Douch and Morun, 1993; Shakya, 2007). The results of this study show that peripheral eosinophilia was more prominent between 3-7 weeks of infection in animals that survived the infections while susceptible animals showed very little increase in eosinophil response at this time. Although, this was not significantly associated with FEC, ( $r = -0.09$ ) in this study, Amarante *et al.* (2005) reported that recruitment of eosinophils, mast cells, globule leukocytes, IgA+ cell counts and histamine concentration were inversely related with *H. contortus* worm burden and FEC and indicated that they had a role in inhibition of development and/ or fecundity of the parasites in resistant Santa Ines breed of sheep. Other workers have also associated eosinophilia with resistance when compared with counts in susceptible sheep infected with *H. contortus* (Stear *et al.*, 2002; Terefe *et al.*, 2005).

Low establishment of parasites, suppression of worm fecundity and the existence of individual variability within and between groups in response to varied levels of *H. contortus* infection, as occurred in this study are believed to be associated with enhanced immune/pathophysiological responses to the infections. The observations by themselves are not direct evidence that the variations are immunologic or genetic in origin but are consistent with parasitological observations from different sheep and goat breeds with *H. contorts* infections in endemic areas and have been exploited in divergent selection studies for resistance to this nematode in sheep.

The application of these findings is that it could be used as a first step in the identification of susceptible animals in a breeding herd and these could then be culled. The mean FEC of individual animals taken between weeks 3-7 after infection, in conjunction with PCV would be a good estimate of the resistance status of the animal. In the derived savannah climate, this could be done at the beginning of the rainy season when there is a moderate challenge of infective larvae. However, this has to be done within a short interval of time since the study indicated that with low infections, egg production and hence pasture contamination could be high. Further selection can then take place based on production traits, so that the most productive animals in the presence of infection can be obtained.

Secondly, selective treatment of individuals with high egg counts can be done without resorting to treatment of the whole flock. This will lead to less pressure on worms and a reduction of the risk of developing anthelmintic resistant worms. These strategies may lead to a reduced cost and increase of profitability for farmers. However, to take full advantage of the within breed variation for selective breeding, more work needs to be done on heritability of this response.

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