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Evaluation of variation in susceptibility of three Ethiopian sheep breeds to experimental infection with *Fasciola hepatica*

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ABSTRACT

A study was conducted to determine the variation in susceptibility of three Ethiopian sheep breeds (Arsi, Horro and Menz) to experimental infection with *Fasciola hepatica*. Arsi and Menz breeds of 16 rams each and Horro breed of 14 rams aged between 6 and 8 months were randomly divided into two groups. All rams in the first groups of each breed were infected with 300 viable metacercariae of *F. hepatica* while those in the second groups were left as controls. Parameters used to assess the level of resistance was fluke egg count (EPG), change in live weight, packed cell volume (PCV) and percentage of adult flukes recovered 18 weeks after experimental infection. The overall mean EPG of Arsi, Horro and Menz sheep breeds was 416.6, 199.1 and 355.7 while the mean number of flukes recovered at the end of the experiment was 107.0 (34.7%), 67.2 (22.3%) and 68.6 (23%), respectively. Based on these factors Arsi breed is ($p < 0.05$) more susceptible to the effect of *F. hepatica* compared to other breeds. The EPG, change in live weight and PCV results showed that Horro breed demonstrated better resistance than the rest groups; nevertheless, in terms of adult parasite recovery, no significant difference ($p > 0.05$) between Horro and Menz breeds was seen. The present results are good indications for the existence of variation in susceptibility of these sheep breeds to infection with *F. hepatica*. Further studies on genetic basis of susceptibility differences needs to be carried out.

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1. Introduction

Fasciolosis is a parasitic disease of cattle, sheep and goats caused by *Fasciola hepatica* and *Fasciola gigantica*. It has a worldwide distribution and is disastrous disease for it causes significant mortality, liver damage and loss of weight (Blood and Radostits, 1994). It is one of the most common helminth infections of sheep in Ethiopia, causing considerable economic losses (Bekele et al., 1992; Yilma and Malone, 1998). The annual loss due to endoparasitism including fasciolosis in Ethiopia is estimated to be 700 million Ethiopian Birr (Mulugeta et al., 1989). Ngategize et al. (1993) estimated the annual economic loss due to ovine fascioliosis in Ethiopian highlands to be 48.4 million Birr.

The use of anthelminthics has almost been the sole control method practiced for some decades in Ethiopia and elsewhere in the world (Baker et al., 1992). However, access to anthelminthics by smallholder livestock producers is often restricted due to high price and scarcity. Even where the anthelmintic drugs are available, they suffer from lack of efficacy against all stages of the parasites. The development of resistance to conventional anthelmintics and the fear of accumulation of anti-parasitic chemical residues in the tissues of host animals are also the other problems associated with the use of anthelmintics (Wooleston and Baker, 1996).

In an attempt to identify alternative methods of internal parasite control that are less dependent on commercial anthelmintics, a number of unconventional methods have

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been reported among which breeding hosts that are resistant to endoparasites has evoked considerable interest. Resistant animals harbor fewer parasites than susceptible animals, which are indicated by their lower EPG and percentage recovery of adult parasites (Wooleston and Baker, 1996). Various reports indicated the presence of substantial variations among sheep breeds in resistance to gastrointestinal parasites, particularly *Haemonchus contortus*, *Ostertagia circumcinta*, and *Trichostrongylus columbriformis* (Baker et al., 1992, 1994). In East Africa (Preston and Allonby, 1979) reported the higher level of resistance of Red Masai sheep to *H. contortus* than Dorper sheep. Genetic variation in resistance to fasciolosis is less documented compared to gastrointestinal nematodes. Some workers have reported the presence of difference in susceptibility among different sheep breeds based on faecal egg output and the percentage of metacercariae that are later recovered as adult flukes (Boyce et al., 1987; Wiedosari and Copeman, 1990). Boyce et al. (1987) pointed out the possible value of selectively breeding for this trait to reduce the production losses caused by fasciolosis. Waweru et al. (1999) comparing susceptibility of Red Masai and Dorper sheep breeds to infection with *F. gigantica* on the basis of egg counts and clinical pathology, reported the Red Masai sheep to be more resistant than the Dorper.

Ethiopia, having diverse climate and topography represents a good reservoir of sheep populations of different genotypes. Arsi, Horro and Menz sheep breeds dwelling in the highland regions of the country represent the three common sheep breeds of Ethiopia (Beyene and Beruk, 1992). The difference in susceptibility of Ethiopian sheep breeds to fasciolosis has not yet been studied. Therefore, this paper reports the results of the study undertaken to explore the difference in resistance/susceptibility to infection with *F. hepatica* among three Ethiopian sheep breeds: Arsi, Horro and Menz.

2. Materials and methods

2.1. Study area and animals

The study was conducted on the premises of the National Animal Health Research Center, Sebeta, Ethiopia. Forty-six rams aged between 6 and 8 months of the three sheep breeds (Arsi = 16, Horro = 14 and Menz = 16) were purchased from the local market in their natural habitat. Arsi sheep breed were brought from Asella, 175 km South of Addis Ababa. Horro breed were brought from Shambu, west Ethiopia 315 km from Addis Ababa while Menz breed were brought from Mehalmeda, 295 km North of Addis Ababa. The animals were ear tagged and acclimatized for 2 months before initiation of the experiment. They were treated for internal parasites with tetramisole (Tetramisole® ERFARs.a., Pallini-Attiki, Greece) and Triclabendazole (Fasionox®, East African Pharmaceuticals, Ethiopia), vaccinated against anthrax, pasteurelosis and sheep/goat pox during the adaptation period. Throughout the study period, the animals were kept indoors on concrete floor house, fed with hay and supplemented with concentrate while water was provided *ad libitum*.

2.2. Experimental design

The randomized block design was employed. Animals from each breed were ranked based on their live weight in ascending order. They were then randomly allocated into two groups, one for treatment and the other for control to minimize variability due to the effect of weight. Each group of Arsi and Menz breeds consisted 8 rams while that of Horro breed had 7 rams.

2.3. Infection

Metacercariae used for this study were produced in the laboratory at Aklilu Lemma Institute of Pathobiology by artificially infecting *Lymnaea truncatula* snails with miracidia hatched from eggs of *F. hepatica* collected from the livers of infected animals slaughtered at Addis Ababa abattoir (Eguale et al., 2006). Metacercariae with transparent cysts and sharply defined structures were selected for infection. The animals in the first group of all breeds were each orally infected with 300 viable metacercariae of *F. hepatica* enclosed in paste formed of concentrate feed. The decision on the dose of metacercariae was reached based on our previous observation where an indigenous sheep infected with higher number was not able to survive longer for collection of data (Eguale and Abie, 2003). The animals in the other groups were left uninfected.

2.4. Post-infection monitoring

Faecal samples were collected from the rectum of each animal every 2-week as of week 10 post-infection and fluke egg count was performed according to Brumpt's sedimentation concentration technique with a little modification (Troncy, 1989). Briefly 1 gm of faeces was homogenized in 10 ml of water, which was sieved with a 1 mm mesh repeatedly by stirring the residue with a glass rod. The suspension was kept for 2 h and the supernatant was decanted. The remaining suspension was centrifuged at 1500 rpm for 3 min. The supernatant was decanted and the water was added to the sediment to get total volume of 2 ml. It was then thoroughly mixed and 0.2 ml of the mixture was rapidly poured into 5 cm diameter petridish with parallel line at its bottom. The number of eggs was counted with dissecting microscope under 40× magnification. Egg per gram of faeces (EPG) was determined by multiplying the number of eggs counted by 10.

Starting from week 0, live weight measurement, packed cell volume (PCV) determination and differential white blood cell (WBC) count were conducted every 2 week. PCV was determined by microhaematocrit method according to Hansen and Perry (1994) and the differential leukocyte counts were conducted by the battlement method on a Giemsa-stained blood smear (Bain et al., 2001).

2.5. Necropsy

Animals died during the course of the trial were subjected to autopsy examination to ascertain the cause of death. The survivors were sacrificed at the end of the experiment on 18-week post-infection to determine the number of flukes in the liver, bile duct and gall bladder. To recover the immature flukes from the parenchyma, the liver was cut into small pieces and suspended in physiological saline at room temperature and it was then gently squeezed. The suspension was strained and the flukes recovered were counted and recorded. In the cases of damaged worms, only the head part was counted so as to avoid repeated counting. Twenty intact worms were randomly picked from each sheep and their length was measured with transparent ruler.

2.6. Statistical analysis

Mean number of adult *F. hepatica* recovered from each experimental group and liver weight after 18 weeks post-infection were compared across experimental groups using one-way analysis of variance (ANOVA). The pattern of EPG, PCV and live weight over time across different experimental groups were examined using line graphs taking variable of interest on the *y*-axis and time on the *x*-axis. For each EPG, PCV and live weight, the change between baseline and overall average of the whole experimental period was also analyzed using ANOVA. Changes in mean values in each outcome (i.e. EPG, PCV and live weight) overtime were compared using repeated measures analysis of variance. Least square difference (LSD) was used to give indication for the treatment group which is significantly different from the rest in case analysis of variance resulted in a globally significant difference among the groups. Results were reported as statistically significant if p-value is less than 0.05.

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