



Comparative Parasitological and Haematological Changes in Two Breeds of Sheep Infected with *Fasciola gigantica*

J.G. Waweru¹, P.W.N. Kanyari^{1*}, D.M. Mwangi¹, T.A. Ngatia¹ and P. Nansen²

¹*Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nairobi, PO Box 29053, Nairobi, Kenya;* ²*Royal Veterinary and Agricultural College, Copenhagen, Denmark*

*Correspondence

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ABSTRACT

Twelve each of Red Masai and Dorper sheep, aged between 6 and 9 months, were acquired from a *Fasciola*-free area of eastern Kenya. Each breed was divided into two groups of 6. The sheep in one group of each breed were experimentally infected with 400 viable metacercariae of *Fasciola gigantica*. The other group of 6 sheep of each breed remained as uninfected controls. The animals were monitored regularly for any evidence of disease. Blood samples taken weekly revealed a general reduction in red cell counts and packed cell volume, which was much faster in the infected Dorper sheep than in the Red Masai. This reduction started from the tenth week after infection and persisted to the end of the experiment 18 weeks post infection (PI). The absolute eosinophil counts rose in all the infected animals, but the values were higher among the Dorper than among the Red Masai. Patency occurred at weeks 12 and 13 PI in the Red Masai and Dorpers, respectively, with the latter shedding significantly more fluke eggs. The worm recovery rates were higher among the Dorpers than among the Red Masai, though not significantly so. On the basis of egg counts and clinicopathology, the Dorper sheep were considered to be more susceptible to *F. gigantica* infections.

Keywords: breed, Dorper, *Fasciola gigantica*, Red Masai, sheep, susceptibility

Abbreviations: PI, post infection; PCV, packed cell volume; RBC, red blood cell; WBC, white blood cell

INTRODUCTION

Fasciolosis is an important cosmopolitan disease of herbivorous domestic and wild animals caused by *Fasciola hepatica* and *Fasciola gigantica*. Mammalian hosts are infected by ingestion of encysted metacercariae and the resulting flukes invade the livers, causing serious losses due to deaths of the hosts, reduced productivity and reproductive efficiency and condemnation of the livers as unfit for human consumption (Ngategize *et al.*, 1993).

Genetic resistance of various breeds of sheep to fasciolosis, as measured by faecal egg output and the percentage of the metacercariae that are later recovered as adult flukes, has been reported previously (Boyce *et al.*, 1987; Wiedosari and Copeman, 1990). The report by Boyce and colleagues (1987) concerned *F. hepatica*, while that by

Wiedosari and Copeman (1990) involved *F. gigantica*. Boyce and colleagues (1987) pointed out the possible value of selectively breeding for such a trait to control this disease. Genetic resistance against gastrointestinal nematode helminths is better documented than genetic resistance in fasciolosis. In East Africa, Preston and Allonby (1978, 1979) reported that Red Masai sheep are more resistant to haemonchosis than Dorper sheep. The current study was designed to compare the susceptibility of Red Masai and Dorper sheep to experimental *F. gigantica* infection using haematological and parasitological parameters.

MATERIALS AND METHODS

Experimental animals

Twelve Red Masai and 12 Dorper sheep, aged between 6 and 9 months, were acquired from an arid fluke-free Eastern province of Kenya and divided randomly into two equal groups, A and B, each group comprising 6 sheep of each breed. The animals were kept indoors throughout the period of the experiment. They were fed on hay and concentrates, with water being provided *ad libitum*.

Infection

The animals in group A were each orally infected with 400 viable metacercariae of *Fasciola gigantica* in gelatin capsules. These metacercariae had been produced in the laboratory by infecting *Lymnaea natalensis* snails with miracidia obtained from *F. gigantica* eggs collected from the livers of infected animals slaughtered in local abattoirs. The animals in group B remained as uninfected controls.

Parasitology and necropsy

Rectal faecal samples were collected once a week and examined for fluke eggs by the method of Boray and Pearson (1960). Any animal that died and the survivors at the end of the experiment were necropsied to recover any flukes from the livers. This was done immediately after death by opening the bile ducts and so recovering the adult worms. To recover the immature flukes from the parenchyma, the liver was cut up into small pieces, about 2 cm square, which were suspended in physiological saline at room temperature and then gently squeezed. The suspension was strained through a tea sieve and the flukes recovered were counted and their lengths were measured by placing them on a ruled glass scale.

Haematology

Following infection, 20 ml of blood from each animal was taken into EDTA by jugular venepuncture at weekly intervals. Red blood cell (RBC) counts and total white blood cell (WBC) counts were determined using an electronic cell counter (Coulter Electronics Inc., 590 W, St Hialeah, FL, USA). The packed cell volume (PCV) was determined by the haematocrit method, while differential white cell counts were done by the battlement method (McGregor *et al.*, 1940) on a Giemsa-stained blood smear. Absolute eosinophil counts were calculated from the differential leukocyte counts and the total leukocyte counts.

RESULTS

Parasitology

Patency occurred at weeks 12 and 13 in the Red Masai and Dorpers, respectively. Following patency, the Dorpers shed more eggs (Figure 1) than the smaller Red Masai sheep so that, at week 14 post infection (PI), the egg counts in the faeces from the former breed was 30 per 5 g of faeces compared to 5 for the latter breed. These differences increased significantly ($p < 0.01$) with time so that by 16 weeks PI the counts were 110 and 10, respectively.

The pattern of fluke recovery for the two breeds is shown in Figure 2. The Dorper sheep suffered higher infestation rates, the livers from the Dorpers yielding more flukes in 83% of the cases. The maximum number of flukes recovered from a Dorper was 95 at 12 weeks PI. With the Red Masai, the maximum number recovered was 74 at 13 weeks PI. The mean number of flukes recovered from the Dorpers was 41.8 (range 15–95) compared with 25 (range 4–74) among the Red Masai, but this difference was not statistically significant ($p > 0.05$).

Haematology

There was a reduction in the PCV and RBC counts in all four groups of sheep (Figures 3 and 4). However, from the ninth week after infection, the infected sheep of both breeds showed significantly ($p < 0.05$) more rapid reductions of both these parameters than the respective uninfected controls.

The eosinophil counts of the infected animals rose from the first week after infection (Figure 5) and persisted up to the 13th and 16th weeks in the Red Masai and Dorpers, respectively. The rise was greater in the infected Dorpers, one of which attained a peak absolute count of 2432/ μl at week 6 PI compared to a peak value of 1330/ μl for the Red Masai sheep ($p < 0.05$).

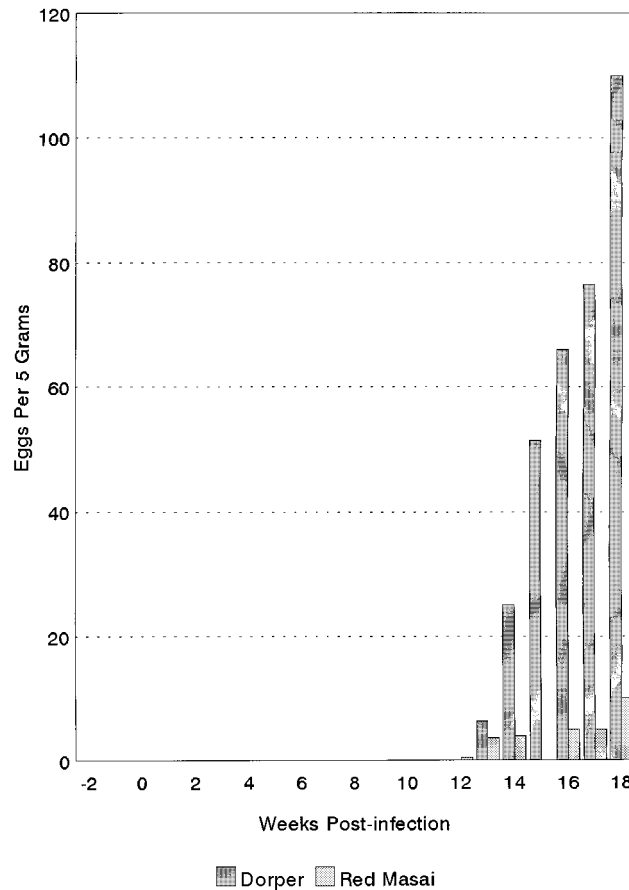


Figure 1. Mean faecal egg counts for the two breeds of sheep

DISCUSSION

There were clear differences in the responses of these two breeds to experimental infection with *F. gigantica* infection. In previously reported studies on genetic resistance in sheep to experimental *F. gigantica* or *F. hepatica* infections, a lower percentage recovery of the metacercariae and lower egg counts in one breed relative to other breeds have been used as indicators of resistance (Boyce *et al.*, 1987; Wiedosari and Copeman, 1990). Boyce and colleagues (1987) recorded fluke recovery rates of 46.0 ± 10.1 in the Barbados Black Belly breed of sheep as opposed to 20.0 ± 20.8 flukes in the more resistant Targhee breed, after infecting both with 500 metacercariae of *F. hepatica*. In 19 Java thin-tailed sheep infected with 500 metacercariae of *F. gigantica*, Wiedosari and Copeman (1990) recorded the recovery of 0–55 flukes from the bile

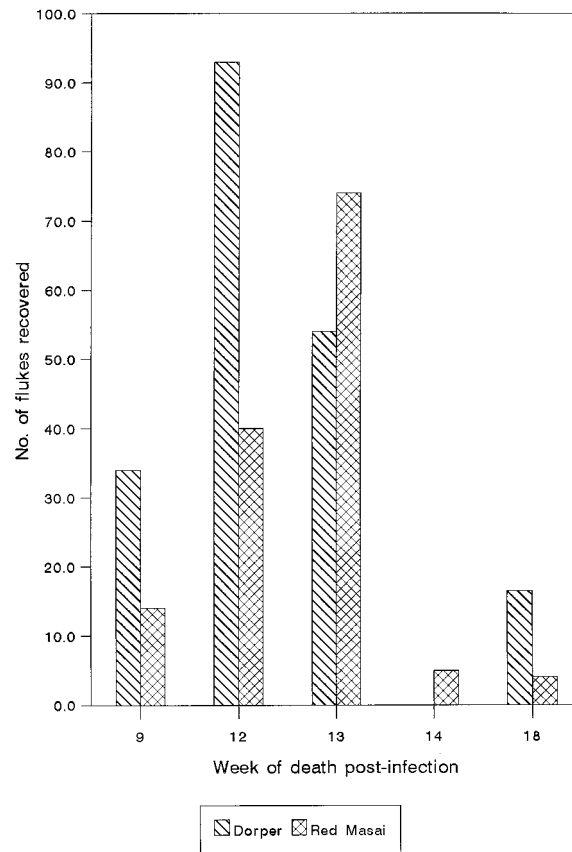


Figure 2. Pattern of fluke recovery from the livers of fluke-infected sheep

ducts after 16 weeks of infection. In the present study, the percentage of the metacercariae that infected the sheep and were later recovered at slaughter was lower in the Red Masai than in the Dorpers. The higher fluke egg counts in the Dorpers may have resulted from the higher fluke burdens but possibly also from higher fecundity of the worms.

The differences in the levels of infection may have a direct bearing on the interbreed differences seen in eosinophil counts. Eosinophilia in helminth infections has been reported to be proportional to the degree of antigenic stimulation or parasitic burden (Ackerman *et al.*, 1981). In the present study, both breeds exhibited eosinophilia from 2 weeks PI. This was more marked in the more heavily parasitized Dorper sheep. Kadhim (1976) also recorded eosinophilia from 2 weeks PI in his studies on experimental *F. gigantica* infections in sheep. The reduction in the number of

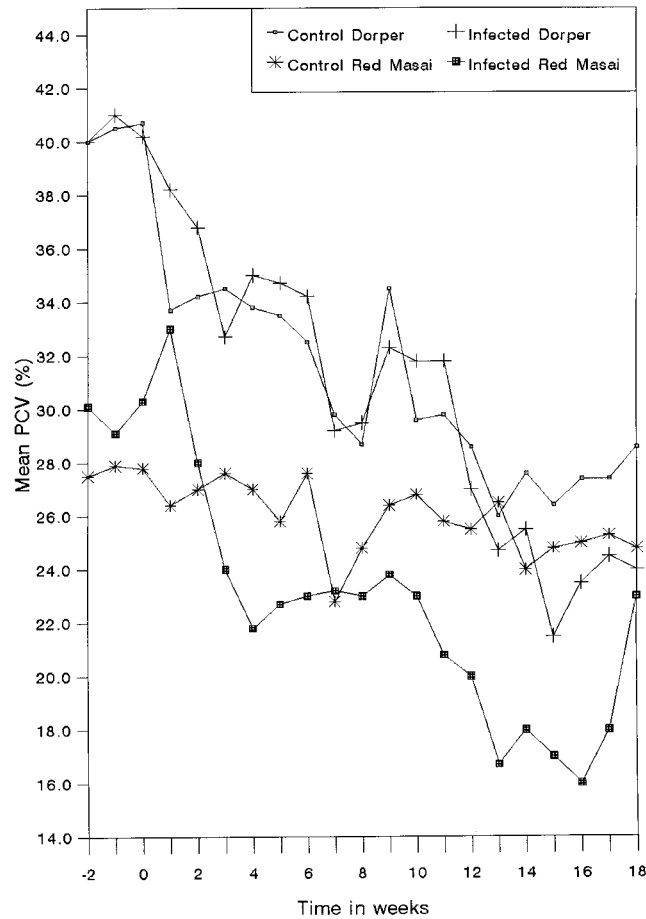


Figure 3. Mean percentage packed cell volume in the two breeds of sheep

eosinophils to the same level as in the control animals by 14 weeks PI in the Red Masai and 16 weeks PI in the Dorpers is in agreement with Sinclair (1967), who found that the eosinophil levels drop back to normal after localization of the flukes in the bile ducts.

The reduced RBC counts and PCV in all the groups may be attributed to the weekly bleeding, stress of confinement and, possibly, to the change of diet from green pasture to dry hay and concentrates. However, the much more marked later loss of blood in the infected animals is to be attributed to the blood-sucking activities of the mature flukes in the bile ducts. The greater loss in the infected Dorpers as compared to the Red Masai sheep will have been due to the higher fluke burden in the former. The marked fall in the RBC counts in the infected animals in the later stages of the experiment

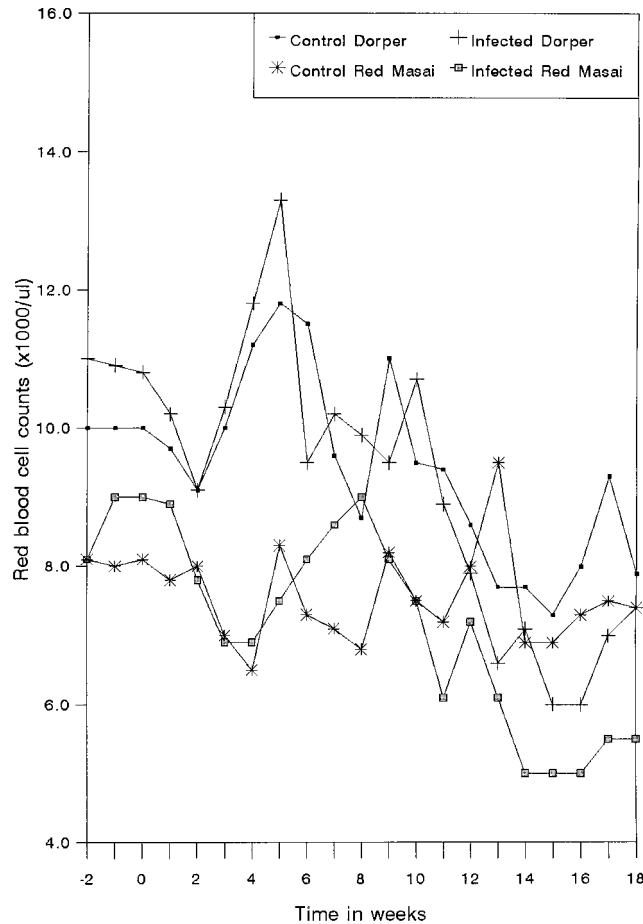


Figure 4. Red blood cell counts in the two breeds of sheep

supports the contention that the anaemia of chronic fasciolosis is mainly due to the haematophagic nature of the parasites in the bile duct (Dargie, 1973). The haematocrit values improved slightly after 16 weeks PI in the present study. Kadhim (1976) recorded a similar phenomenon in ovine fasciolosis but did not venture to explain the cause.

These findings suggest that the Red Masai are more resistant than Dorper sheep to a primary infection with *F. gigantica*. Although the number of animals used in this study was low, the results form an indicative basis for a wider study under natural field conditions, involving larger numbers of sheep. Other breeds of sheep could also be studied to assess their relative status to fasciolosis.

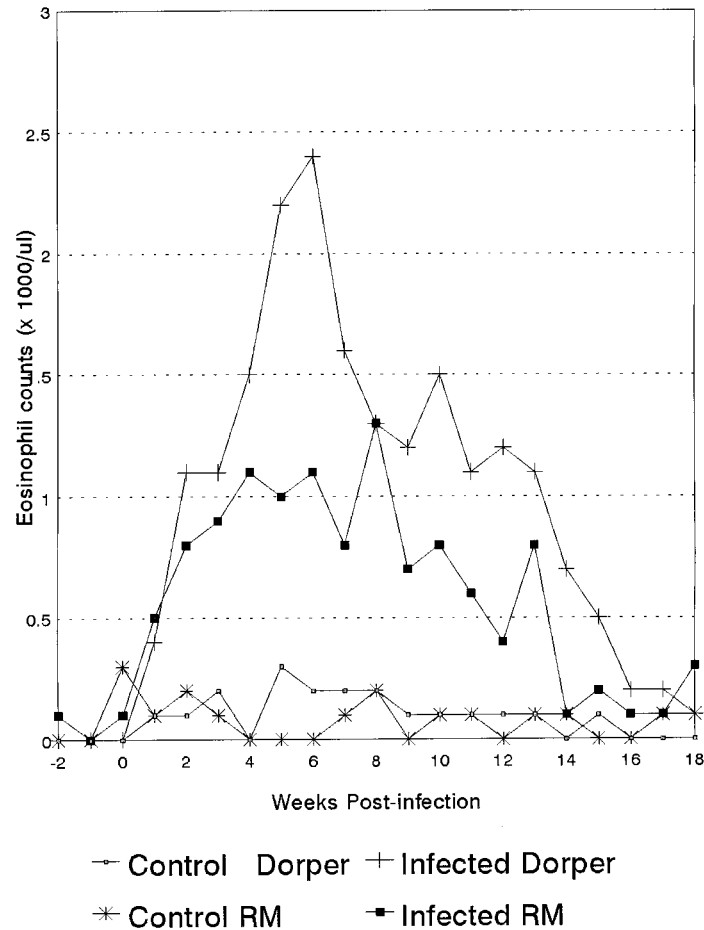


Figure 5. Absolute eosinophil counts in the two breeds of sheep

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Comparaison des changements hématologiques et parasitologiques chez deux races de mouton infectées avec *Fasciola gigantica*

Résumé – Douze animaux de la race Red Masai et Dorper âgés entre 6 et 9 mois furent achetés dans une région de l'est kenyan où il n'y a pas de cas de fasciola. Les animaux d'une même race furent divisés en 2 groupes de 6. Un groupe pour chaque race fut infecté avec 44 metacercaires vivantes de *F. gigantica*. L'autre groupe ne fut pas infecté et servit de témoin. Les animaux furent suivis régulièrement pour le suivi de la maladie. Des échantillons prélevés hebdomadairement montrèrent une réduction générale des globules rouges et de l'hématocrite de façon plus rapide chez les moutons infectés de la race Dorper que chez ceux de la race Red masai. Cette diminution commença à la dixième semaine après l'infection et se poursuivit jusqu'à la fin de l'expérience à savoir 18 semaines après l'infection. Le nombre d'éosinophiles augmenta chez les 2 groupes infectés mais il fut plus élevé chez la race Dorper que chez la race Red masai. La race Dorper élimina plus de kystes (de façon significative) et de vers adultes (non significatif) que l'autre race. Sur la base du nombre de kyste et des résultats de pathologie clinique les moutons de la race Dorper furent considérés comme étant plus ecclin à l'infection par *F. gigantica*.

Cambios comparativos parasitológicos y hematológicos en dos razas de ovejas infectadas con *Fasciola gigantica*

Resumen – Doce ovejas de raza Red masai y doce de raza Dorper de entre seis y nueve meses, han sido adquiridas en un área libre de *Fasciola* en el este de Kenia. Cada casta se ha dividido en dos grupos de seis. Las ovejas de un grupo de cada raza han sido experimentalmente infectadas con 44 viables metacercariae de *Fasciola gigantica*. El otro grupo de seis ovejas de cada raza ha permanecido como grupo control no infectado. Los animales se han monitorizado regularmente para cualquier evidencia de la enfermedad. Las muestras de sangre tomadas semanalmente revelan una reducción general de glóbulos rojos y de volumen celular, que ha sido mucho mayor en las Dorper infectadas que en las Red masai. Esta reducción empieza

en la décima semana tras la infección y persiste hasta el final del experimento, 18 semanas post-infección (PI). El recuento absoluto de eosinófilos aumenta en todos los animales infectados pero los valores son más altos en las Dorper que en las Red masai. Aparece patency en las semanas 12 y 13 PI en las Red masai y en las Dorper respectivamente, con las últimas desprendiéndose significativamente de más fluke eggs. Los niveles de recuperación definitiva han sido mayores en las Dorper en comparación con las Red masai, aunque las diferencias no han sido significativas. En base al recuento de huevos y a la clinicopatología, la oveja Dorper se considera más susceptible a las infecciones de *Fasciola gigantica*.