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Haematological changes due to bovine fascioliasis

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This study determined the haematological changes due to the infection of fascioliasis in cattle. The haematological indices of blood samples collected from purposely selected *Fasciola*-infected and non-infected cattle were analyzed using standard methods. Statistical analysis revealed high significant differences between the packed cell volume (PCV), haemoglobin (Hb) and red blood cells (RBC) of infected and non-infected cattle (p<0.05). Significant differences existed between the white blood cells (WBC), mean cellular volume (MCV) and mean cellular haemoglobin (MCH) of both groups (p<0.05). No significant difference was observed between the mean cellular haemoglobin concentration (MCHC) of the infected cattle and the control. There was notable reduction in PCV, Hb and RBC with increase in worm load and a multiple regression analysis revealed significant negative correlation between worm load and RBC, Hb and PCV with correlation coefficient values, r = -0.616, -0.592 and -0.615, respectively. Levels of neutrophils, eosinophils, monocytes and lymphocytes increased progressively as worm load increased. Only basophils showed no change. Multiple regression analysis confirmed a statistically significant positive correlation between eosinophils and worm load (r = 0.575) and between neutrophils and worm load (r = 0.601). Lymphocytes had no significant positive correlation with worm load (r = 0.070), while monocytes had no significant negative correlation with worm load (r = - 0.062). The implications of the above findings are discussed.

Key words: Fascioliasis, haematology, haemoglobin, neutrophils, eosinophils, monocytes.

INTRODUCTION

Bovine fascioliasis is a systemic infection of cattle caused by the liver flukes, *Fasciola gigantica* and *Fasciola hepatica*. It is an important and well established veterinary disease of both domestic and wild ruminants such as cattle, goats, sheep and swine, causing enormous economic losses in livestock industries through animal mortality, growth retardation, sterility, condemnation of affected livers and expense due to control measures (Malone et al., 1998). Fascioliasis is a secondary zoonotic infection in man. The World Health Organization (WHO) listed it among human parasites of public health importance (WHO, 2007). Studies have shown that human fascioliasis has increased significantly in 51 countries of the world since 1980 with several geographical areas being highly endemic with the disease (Mas-coma et al., 1999).

In endemic regions, the prevalence in humans is high as 65 to 92% in Bolivia and in animals as high as 89.5, 44.8 and 39% in South Cotabato, Philippines (Adedokun et al., 2008) and Nsukka, eastern Nigeria (Ikeme and Obioha, 1973). In all, it is worth noting that fascioliasis in every endemic region of the world causes serious clinical

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implications and economic losses.

It has been estimated that internal parasites cost the livestock industry in excess of US$2 billion per year in lost productivity and in increased operational expenses (Axford et al., 2000). Some helmintic infections like liver fluke are characterized by the reduction of voluntary food intake and a decrease in efficiency in the use of these feeds by the infected animals (Amarante, 2001; Keyyu et al., 2005; Muturi et al., 2005). They, therefore, have a negative effect on animal growth and production (Kahn et al., 2000), and proper body functioning. The most common genera of trematodes causing reduced animal growth and production is *Fasciola* which induces resilience and mal-functions of certain organs and systems in the body. Resilience and body mal-functions are based on the inability of the animal to withstand the pathogenic consequences of an endoparasite infection and this can be evaluated by observing the haematological parameters and biochemical indices (Keyyu et al., 2005). The haematological tests can help to indicate the effect of disease on the blood components. Bovine fascioliasis can result in significant blood losses with all associated consequences (Soun et al., 2006), although adverse effects depend on the parasite load (Coop and Kryziakis, 2001; Wiedosari et al., 2006).

F. hepatica or *F. gigantica* migrates through the liver parenchyma and tissue and when mature feeds on the blood of the final host at a rate of 0.2 to 0.5 ml per day per fluke (Wiedosari et al., 2006), which leads to severe anaemia. As such, determination of the concentration of blood parameters (erythrocyte counts, white blood cell counts, pack cell volume, haemoglobin concentration and the differential counts) for cattle will provide information that serves as the basis for the diagnosis, treatment and prognosis of the diseases that could affect them (Yokus and Cakir, 2006).

Few studies have documented the haematological alterations due to naturally acquired bovine fascioliasis. We therefore investigated the haematological changes in cattle due to bovine fascioliasis using cattle with naturally acquired fascioliasis and without other diseases using direct organ and tissue analysis. Also, the diagnostic procedure used is very reliable and more readily affordable in endemic low-resource rural communities.

MATERIALS AND METHODS

Collection of blood samples

Jugular blood samples were collected from 57 cattle with naturally acquired bovine fascioliasis and no other disease out of 659 cattle and 20 non-infected cattle within Nsukka tropical ecosystem in southeast Nigeria. The selected slaughtered cattle were confirmed free from other possible diseases through visual inspection of the organs, intestine and tissues by qualified veterinary officers. Two milliliters of the jugular blood samples collected from each animal sample was dispensed into evacuated EDTA tubes and mixed properly. These were stored at 4°C according to Coles (1986) and used within 12 h for the haematological studies.

Haematological studies

The haematological indices of the collected blood samples were analyzed using standard methods. Haemoglobin (Hb) concentration was determined using the cyanomethaemoglobin method (Blaxhall and Daibley, 1973), total erythrocyte and leucocytes (RBC and WBC) counts were done using an improved Neubouer haemocytometer. The packed cell volume (PCV) was determined using the microhaematocrit centrifuge technique (Dacie and Lewiz, 1984).

The mean cellular haemoglobin (MCH), mean cellular volume (MCV) and mean cellular haemoglobin concentration (MCHC) were evaluated from the results of RBC, WBC, Hb and PCV according to the methods given by Baker et al. (1975). The differential leucocytes count was conducted as described by Coles (1986).

Ethical approval

The ethical requirements of the University of Nigeria involving research with livestock was fully complied with.

Analysis of data

The data obtained was analyzed using SPSS version 16. The student t-test was used to analyze the significant differences between the haematological parameters of the *Fasciola*-infected and the non-infected samples. Values of $P<0.05$ were considered significant. Results were expressed as means ± SD. Correlation between worm load and haematological values were tested using regression analysis.

RESULTS

Haematological profile

The results of the haematological indices determined for *Fasciola*-infected and non-infected cattle are presented in Table 1. The result indicated that PCV, Hb and RBC were lower in the infected cattle than in the control and on the other hand, the WBC, MCHC, MCH and MCV were higher in the infected cattle than in the control. Statistical analysis revealed high significant differences between the PCV, Hb, and RBC of the infected and non-infected cattle ($p<0.05$) and also significant differences existed between the WBC, MCV and MCH of both groups ($p<0.05$). No significant differences occurred between the MCHC of the infected cattle and the control.

The differential counts of *Fasciola* infected cattle and the control

The result of the differential counts of the infected cattle

Table 1. The mean values of the haematological parameters of *Fasciola* infected and the uninfected cattle (±SD).

Haematological Parameters	Infected	Range	Uninfected	Range
PCV (%)*	24.13 ± 0.71	19.5 – 29	37.35 ± 1.09	30 – 50
Hb (g/dl)*	7.42 ± 0.20	5.7 – 9.6	11.13 ± 0.27	8.5 – 13.6
RBC (cells/mm^3) x 10^6*	3.86 ± 0.13	3.0 – 4.89	7.23 ± 0.23	5.51 – 8.9
WBC (cells/mm^3)*	19995 ± 6457.14	9600 – 17500	8750 ± 443.76	6300 – 12100
MCHC (%)	30.85 ± 0.58	28 – 36	29.55 ± 0.52	26 – 33
MCH (pg)*	19.50 ± 0.35	17 – 22	15.10 ± 0.38	12 – 18
MCV (μm^3)*	62.65 ± 0.51	61 – 69	51.55 ± 0.64	46 – 56

*Significantly different between infected and uninfected cattle.

Table 2. The mean values of the differential count of *Fasciola* infected and the uninfected cattle (±SD).

Differential counts (%)	Infected	Range	Uninfected	Range
Neutrophils*****	43.15 ± 1.27	36 – 59	34.27 ± 1.02	26 – 40.5
Eosinophils*****	4.00 ± 0.40	2 – 8	0.77 ± 0.49	0.49 – 1.11
Monocytes*****	1.69 ± 0.17	0.4 – 3	4.41 ± 0.50	0.83 – 8.58
Basophils*****	00	00	00	00
Lymphocytes*****	44.05 ± 2.61	29 – 68	52.65 ± 0.86	45 – 63

*Significantly different between infected and uninfected cattle.

and the uninfected are recorded in Table 2. There was a noticeable increase in the neutrophils and eosinophils of the infected cattle and a clear decrease in the level of monocytes and lymphocytes in the infected cattle. No difference was observed in the values of basophils in both groups. The differences in the neutrophils, eosinophils, monocytes and lymphocytes of both groups were highly significant at $P<0.05$.

The effects of worm load on haematological indices

The PCV, Hb and RBC of *Fasciola* infected cattle with the corresponding worm load is shown in Figure 1. The result showed a notable reduction in PCV, Hb and RBC with increase in worm load. Multiple regression analysis revealed significant negative correlation between worm load and RBC, Hb and PCV with the correlation coefficient values, r = -0.616, -0.592 and -0.615, respectively. Some increase in WBC was observed with increase in worm load (Figure 2). The analysis expressed significant positive relationship between worm load and WBC (r = 0.737). MCV had slight increase with increase in worm load, while MCHC and MCH showed no reasonable change with increase in worm load (Figure 3). MCV had positive relationship with worm load although not significant (r < 0.5).

The effects of worm load on the differential counts

The result of the differential counts against *Fasciola* worm load is shown in Figures 4 and 5. From the result, it could be deduced that neutrophils, eosinophils, monocytes and lymphocytes increased progressively as the worm load increased. Only basophils showed no change. Multiple regression analysis confirmed significantly positive correlation between eosinophils and worm load (r = 0.575) and between neutrophils and worm load (r = 0.601) as shown in Figure 4. Lymphocytes had no significant positive correlation with worm load (r = 0.070) while monocytes had no significant negative correlation with worm load (r = - 0.062).

DISCUSSION

Diagnostic methods have been serious issues in the treatment and control of diseases. Such techniques have been very important for identifying the aetiologic factors, disease patterns and effects which play a major role in the control of such diseases and in public health decision

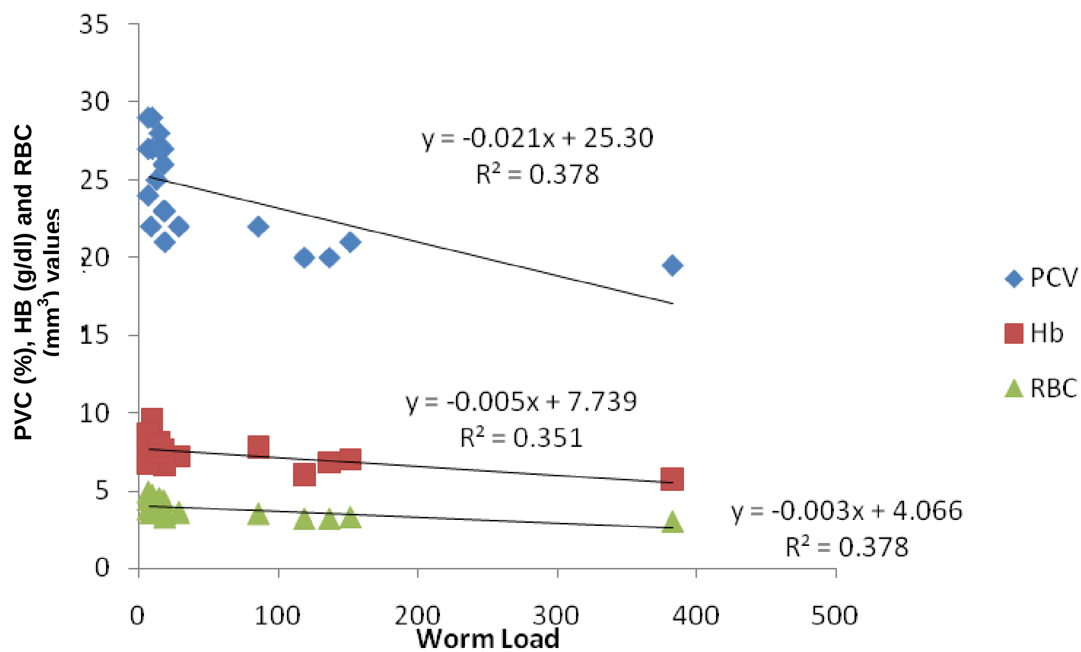

Figure 1. The linear regression of PCV, Hb and RBC against worm load.

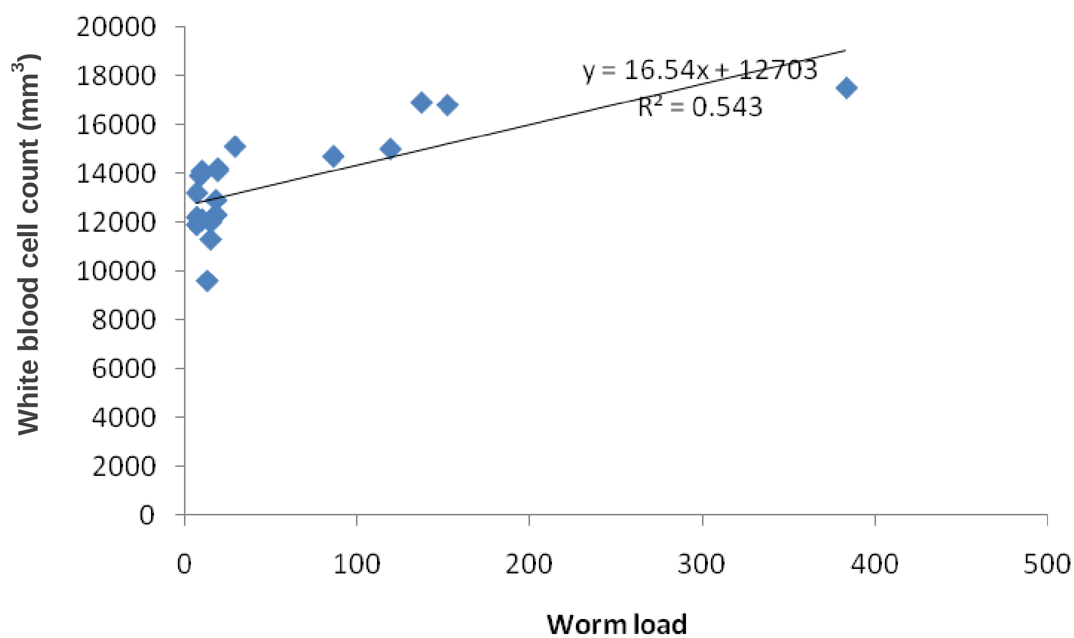

Figure 2. The linear regression of WBC against worm load.

making (Downs, 1990; Habbari et al., 1999). To this effect, this study encompasses current information on bovine fascioliaisis and the consequent haematological changes due to infection of the disease. It serves to provide data for the disease diagnosis and control, and for health services planning in both cattle and human populations.

It was observed that high significant decrease existed

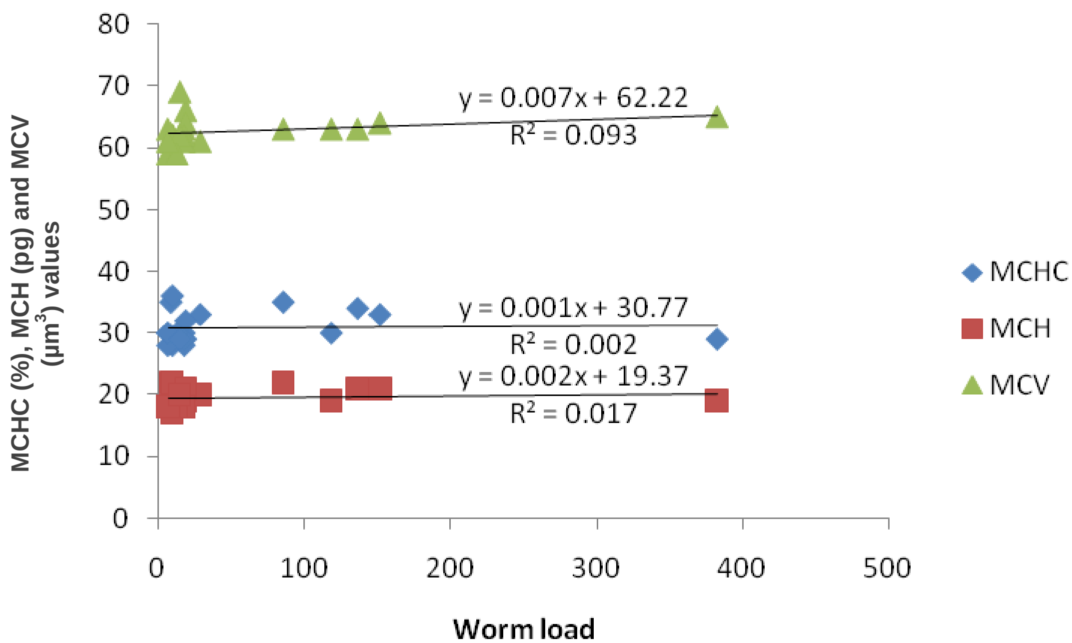

Figure 3. The linear regression of MCV, MCH and MCHC against worm load.

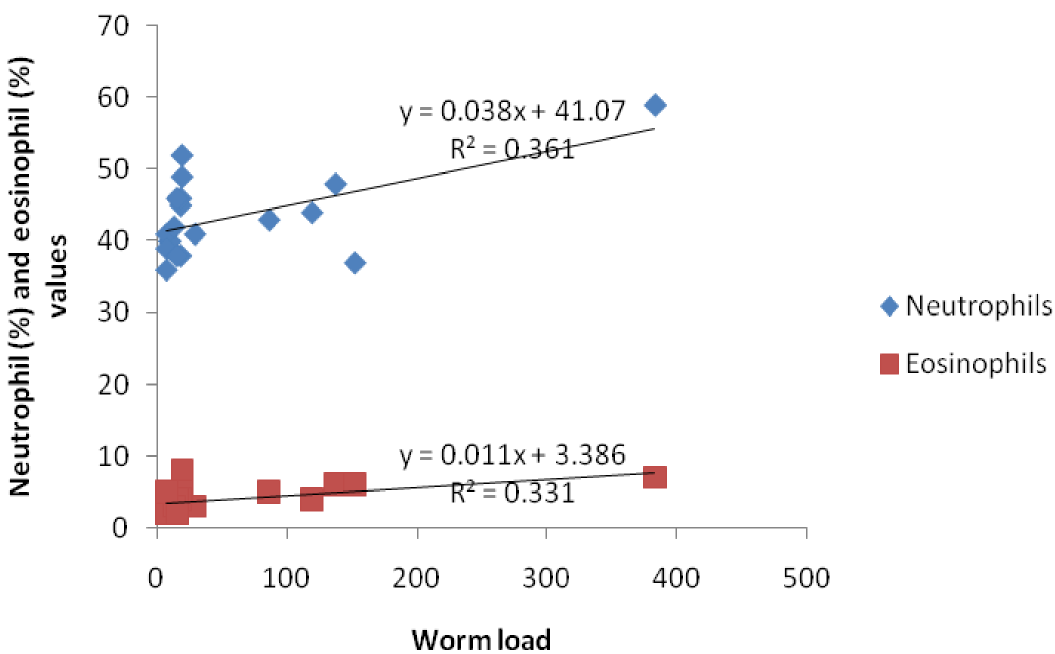

Figure 4. The plot of neutrophil and eosinophil levels against worm load.

in the PCV, Hb and RBC of *Fasciola* infected cattle when compared with those of the control, thereby indicating a normochromic and normocytic anaemia. The results for PCV and RBC are similar to those found by Molina et al. (2006) in cattle, while the results for RBC and Hb were similar to those reported by Haroun and Hussein (1975) in cattle and by Doaa et al. (2007), Waweru et al. (1999 and El-Aziz et al. (2002) in sheep. Ahmed et al. (2006)

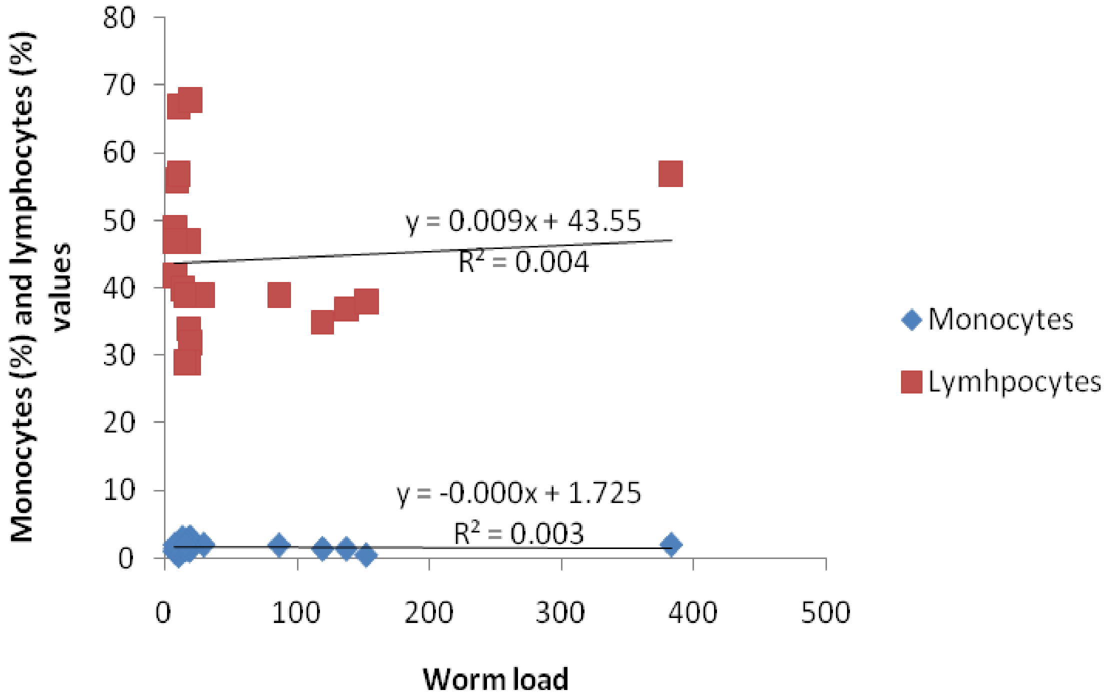

Figure 5. Plot of monocytes and lymphocytes levels against worm load.

and Matanović et al. (2007) had similar observations in PCV, Hb and RBC in sheep with fascioliasis. Also, Sykes et al. (1980) found significantly lower RBC and haemoglobin concentrations together with higher MCV during chronic subclinical fascioliasis in sheep. The reduction in RBC counts, Hb and PCV in this study may be attributed to the acute loss of blood caused by the flukes or extensive loss of blood into bile duct due to the large amounts of flukes present in the liver. Kaneko et al. (1997), Kramer (2000) and Lotfy et al. (2003) also reported that the severe anaemia may be due to a chronic liver inflammation, which causes depression of erythrogenesis. This reduction was also found to be inversely proportional to worm load, which is in line with the findings of Rowland and Clampitt (1979), who reported that there is a good correlation between the level of infestation and erythrocyte numbers, packed cell volume, haemoglobin concentration and plasma protein values. Hawkins (1984) also suggested haemoglobin and PCV to be useful in predicting the size of fluke load and in indicating the likelihood of death or survival of infected sheep. On the other hand, significant increase was obtained in WBC, MCV and MCH of the infected cattle, when compared with those of the control group. Sykes et al. (1980) and Haroun and Hussein (1975) also observed similar higher MCV in the infected animals. In the present study also, infection with *F. gigantica* caused marked neutrophilia, eosinophilia, monocytopenia and lymphocytopenia. Thus, the total neutrophils and eosinophils counts were significantly higher in the infected group when compared with the control, and the monocytes and lymphocytes levels were significantly lower in infected groups than in the control. Haroun and Hussein (1975) reported the same eosinophilia, monocytopenia and lymphocytopenia in infected cattle, though with a reduction in neutrophils. Similar results were also recorded in sheep by Waweru et al. (1999) and El-Sayed et al. (2003). The changes in the differential counts may be a means of body defense against *Fasciola* obstructive effects or due to the toxin mediated lesion of the bone marrow (Penny et al. 1996). The neutrophilia and eosinophilia were also observed to be proportional to worm load. Widjajanti et al. (2002) equally observed a positive correlation between eosinophil counts and the number of liver flukes recovered. This may be due to inflammation and infection resulting from the activity of the flukes in the bile ducts as mentioned by Radostits et al. (2000). Moreover, eosinophilia has been reported to be proportional to the degree of antigenic stimulation or parasitic load in helminth infections (Ackerman et al. 1981). This is normally linked to antigen antibody reaction which occurred when the sensitivity to the protein of the parasites has developed or when the secretory products were released within the blood (Jain, 1993) associated with cellular-mediated immunity (Duffus et al., 1980). In addition, monocytopenia might be due to increased chemotaxis to the inflammatory process in the bile ducts (Coles, 1986).

Summary and conclusion

The haematological assay in this study showed reduced RBC, Hb and PCV and eeosinophilia, nuetrophilia, monocytopaenia and lyphocytopaenia in the infected cattle and they are noticed to be significantly correlated to worm load. It could be concluded that changes in the haematological parameters in cattle infected with *F. gigantica* reflect tissue damage. The degree of the changes in the haematological parameters and tissue damages are also dependent on the infective dose of metacercariae. The effects of *F. gigantica* on the haematological values were noticed even in the lowest worm load, suggesting that monitoring their levels in farms could be useful in early diagnosis and prognosis for cattle fascioliasis. With the early diagnosis of fascioliasis in animals, a treatment schedule could be designed to avoid more infection and animal losses at the farm level and in turn economic losses will be appropriately reduced.

Finally, even though human fascioliasis had gained awareness in some endemic countries like Chile and Egypt, in some countries like Nigeria, the awareness of the disease is still at the lowest ebb and there is much possibility of many unnoticed and even fatal cases in the rural and urban areas resulting from wrong diagnosis and lack of awareness of the disease both within the medical sectors and the society. As such, deployment of haematological tests can aid and enhance effective diagnosis of the disease.

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