Evaluation of *Fasciola gigantica* infection by serial enzyme patterns

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SUMMARY

Three calves were infected with 2000, 3000 and 4000 metacercariae of *Fasciola gigantica*. Two other calves were left as uninfected controls. All calves were kept indoor for 23 weeks. During this period, serial samples of plasma and faeces as well as body weight gains were taken. Eggs were first detected in the faeces of infected calves on week 13 post infection (PIW13). In the infected animals, AST activity rose from an average level of 57.5 units/ml to 71 units/ml whereas GGT rose from an average level of 7.8IU/l to 65.6 IU/l. The percentage increase in body weightS of all calves were comparable during the first nine weeks after infection. Between PIW 9 and 13, the infected animals gained weight between 0.6% and 0.7% of their initial body weights. The percentage increase in body weights of the controls during the same period was between 11% and 17%. It was concluded that serial plasma enzyme patterns are useful in evaluating the different stages of *F. gigantica* infections.

Key words: Liver flukes, *Fasciola gigantica*, GGT, AST, liver enzymes

INTRODUCTION

Fasciolosis is a zoonotic helmintic infection caused by trematodes in the family Fasciolidae (Soulsby, 1982). The commonly known species of Fasciolidae in tropical and subtropical countries is *Fasciola gigantica* whereas *Fasciola hepatica* is almost entirely found in countries with cold and temperate climates (Soulsby, 1982). In many tropical countries information about *F gigantica* infection in animals is derived from abattoir records of slaughtered animals, particularly bovine (Hyera, 1984). Although data from the abattoir is useful for identification of problem areas and may be used to reveal the prevalence, seasonal variation and economic importance of the disease, it does not avail any information regarding the development of the disease in live animals. In order to achieve this important information, it is necessary for other diagnostic methods to be applied.

Currently, the most used diagnostic method in tropical countries is examination of faeces for the presence of fluke eggs. This method however, has a serious drawback because the infection cannot be diagnosed until liver damage associated with the migration of juvenile forms of the flukes has occurred and the flukes have established themselves in the bile ducts. Furthermore, because of day-to-day variation in egg count and a maximum rate of egg recovery of only 45-60% (Boch and Supperer, 1983), the method precludes quantitative diagnosis.

In view of these shortcomings methods involving examination of blood parameters are recommended (Wyckoff and Bradley, 1985; Pfister *et al.*, 1986). Most work on methods using blood parameters has been reported on *F hepatica* infection and has focused mainly on the determination of enzymes particularly aspartate aminotransferase (AST) and gamma-

glutamyltranspeptidase (GGT) (Simesen *et al.*, 1973; Wyckoff and Bradley, 1985). This work was to investigate the practical value of serial AST and GGT determinations in the diagnosis and evaluation of experimental *F gigantica* infection in cattle.

MATERIALS AND METHODS

Site of the experiment

The experiment was conducted at the Sokoine University of Agriculture Farm, Morogoro, Tanzania which lies approximately latitude 6°50'20" south and longitude 37°39'20" east at an altitude of 528 m above see level. The annual rainfall is about 800 mm.

Experimental animals and management

Five Jersey-Friesian cross bred bull calves aged between 6 and 15 months and weighing an average of 162 kg were randomly divided into two groups of three and two. Calves in group 1 (606, 618 and 2854) were earmarked to be infected while those in group II were to be uninfected controls. Prior to the beginning of the experiment, all calves were isolated from other animals and collectively kept indoors on a concrete floor which was washed every day. They were maintained on silage and hay and supplemented with a concentrate ration at the rate of 1.5 kg per animal. Water was given to the animals *ad libitum*. Three consecutive monthly faecal sample examinations were carried out on each calf. Thick and thin blood smears were made from the peripheral blood to check for possibility of tick-borne infections. In an attempt to eliminate incubating infections all calves were given therapeutic doses of oxytetracycline followed two days later by treatment within an antitryranosomiasis drug Diminazine aceturate (Berenil). The calves were also drenched once with Thiophanate (Nemafax) against helminthiasis.

Infection by metacercariae

F. gigantica metacercariae were produced in laboratory snail cultures of *Lymnaea natatensis* at Muguga, Kenya. Prior to dosing of the calves, the viability of the metacercariae was checked by stimulation of the metacercarial by artificial digestion (Wikerhauser, 1960). Three single doses of 4000, 3000, and 2000 metacercariae were separately counted under a dissecting microscope, wrapped in filter papers and administered to calves 606, 618 and 2854 respectively on first week (zero post infection week (PIW)). Administration of the doses was done by placing the wrapped filter paper in a balling gun, pushing it into the mouth at the base of the tongue and forcing the animal to swallow it by drenching with 500 ml water. The remaining two calves 619 and 2859 were made to swallow a wrapped filter paper containing no metacercariae in a similar way.

Measurement of body weights and sampling of faeces and blood

The initial body weights of calves 606, 618, 619 and 2859 were taken on zero (PIW). Subsequent measurements were done on the fourth PIW and fortnightly thereafter up to PIWI8. Body weight measurements for calf 2854 missed this consistence and were, therefore, not included. The obtained body weight gains for each calf were expressed in percentage of their initial body weights. Rectal faecal samples were daily taken from all calves beginning on zero PIW until the eggs were first seen in the faeces. Thereafter sampling was done weekly.

All the animals were bled from the jugular vein using sterile stainless - steel needles

zero PIW. Thereafter the animals were bled at weekly intervals up to PIW 23. A whole blood sample from each animal was taken in a vacutainer containing heparin and centrifuged at 3000 rotations per minute (rpm) to obtain plasma. Each plasma sample was aspirated in two separate 2.0 ml aliquots and frozen at -20°C before analysed for AST and GGT.

Analytical Methods

AST was determined by the colorimetric end point method of Reitman and Frankel (1957). A mixed series of six increasing concentrations of pyruvate (0.0 - 1.0µM) and corresponding series of six decreasing concentrations of 2-oxoglutarate (2.0-1.0µM) were prepared such that the total concentration of pyruvate and 2-oxoglutarate in each mixture was always 2µM. A standard curve for pyruvate based on the colorimetric readings of increasing concentrations of pyruvate was prepared and checked for linearity prior to preparation of an AST calibration curve by plotting the pyruvate concentration against corresponding AST units established by baker (1968). During analysis, the test sample and plasma blanks were incubated at 40°C for 60 minutes. The colorimeter reading of the plasma blank was subtracted from that of the test and the difference was converted into AST units using the established AST calibration curve.

GGT was determined with reagents prepared according to the colorimetric method of Jacob (1970). L-gamma-glutamyl-p-nitroanilide was used as substrate after being dissolved with glyclglcine and $MgCl_2$ in ammediol - HCl buffer. The p-nitroaniline which has a maximum absorbance at 405±10 run was used as an indicator to detect the enzyme activity after stopping the reaction by addition of 0.0075N NaOH. The procedure of Jacob (1970) was modified in this study by only doubling the incubation time at 37°C to 90 minutes so as to allow samples with low activity to give sufficient colorimetric readings. Faecal samples were analysed for fasciola eggs according to the sedimentation method described by Price and Reed (1970). The results were analysed statistically using StatView® software.

RESULTS

Enzymological changes

AST values in the controls varied from 50 to 65 units/ml during the 23 weeks' experimental period. The calculated average value in the controls was 57.5 (48 determinations). In the infected calves, the values of AST were similar to those in the controls up to PIW 6 (Figure l). Thereafter the values gradually rose from around 60 to 110 units/ml by PIW 11- 12. Other peak values of 84 and 95 units/ml occurred at PIW 16 and 22 respectively. The calculated average value in the infected animals of 7lunits/ml (72 determinations) over the 23 weeks experimental period was significantly different ($P<0.05$) from that in the controls.

GGT values in the controls varied from 5 to 9IU/l during the 23 weeks experimental period. The calculated average value was 7.8IU/1. In the infected calves the values of GGT were similar to those in the controls up to the PIW 10-11. Thereafter the values rapidly rose around PIW 12-16 to three different peak values (Figure 2) of between 15 and 39 times the activity in the controls. After the peak levels, the activities decreased to levels which were still between 5 and 30 times the activity in the controls by the end of the experiment. The calculated average value in the infected animals of 65.6IU/l differed significantly ($P<0.01$) from that in the controls.

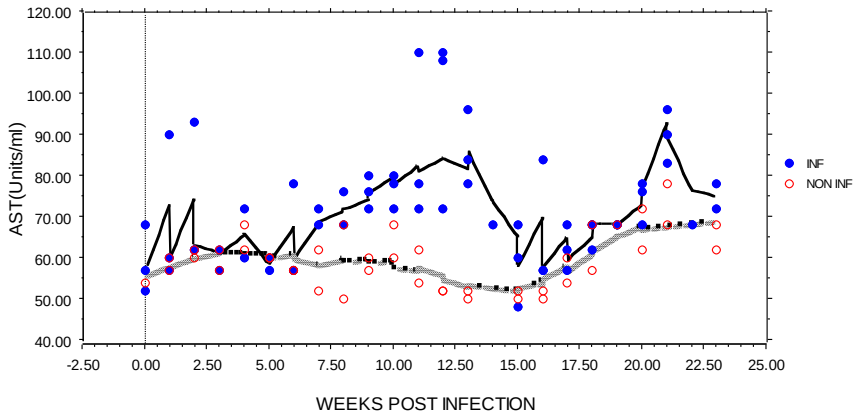

Figure 1. AST values

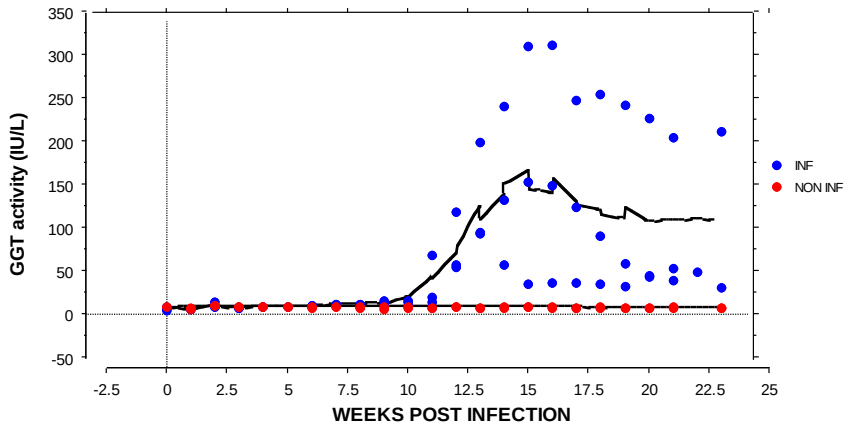

Figure 2. GGT activity

Body weight gains

The percentage increase in body weights of controls and infected animals were comparable during the first 8 weeks of the experiment (Figure 3). Thereafter the percentage increase in the infected animals slowed down to only between 0.6 and 0.7% of their initial body weights over a period of four weeks (PIW 8-12). During the same period the controls gained between 11 and 17% of their initial body weights. There was a 2-6% increase in the body weights of infected animals between PIW 12 and 18 which was still for below the 8-13 % increase recorded in the controls over the same period. The calculated mean values of % increase in the controls and infected animals differed significantly ($P<0.05$) from each other.

Faecal egg counts

*F. gigantica*_eggs were first seen in the faeces of infected animals by PIW 13 (Figure 4). The egg count per gram (EPG) in the three infected animals rose steadily and rapidly between PIW 14 and 18 after which it varied and showed a tendency to decline.

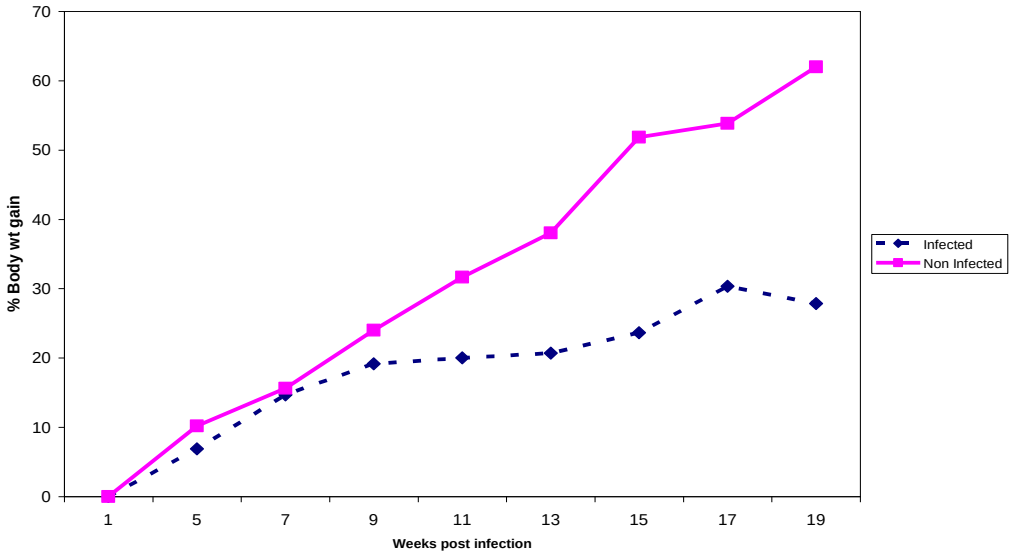

Figure 3. Percentage increase in body weights

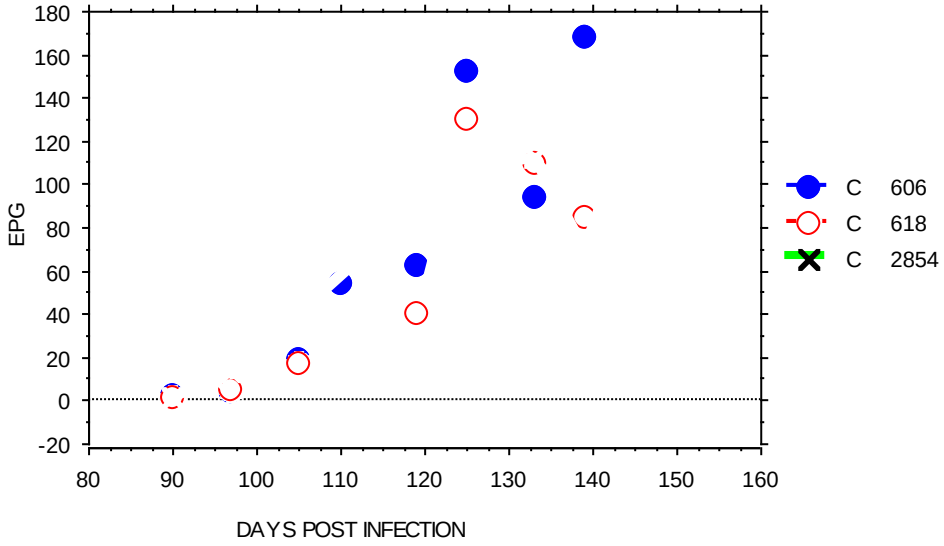

Figure 4. Faecal egg counts per gram

DISCUSSION

The results of the present study indicate that the conventional method of diagnosing fasciolosis by detection of fluke eggs in the faeces was positive at PIW 13 (Figure 4). The pre-patent period of 91 days observed in the present study is in agreement with that reported by other workers in cattle infected by *F. gigantica* (Sewell 1966; Bitakamire and Bwangamoi, 1969; Hammond and Sewell, 1974). However, the period is longer than that of 70 days reported in cattle infected by *F. hepatica* (Balbo *et al*., 1973; Wyckoff *et al*., 1985).

In the present investigation, AST activity in infected animals began and continued to deviate from those in the controls from 6 to

12 PlW (Figure l). Although increases in AST activity may also be associated with cellular damage to tissue other than the liver such as skeletal and cardiac muscle (Cornelius, 1980), observation of significantly higher plasma AST activity in infected calves than in the controls during the later part of the migratory phase (PlW 13) suggests that the increases in AST activity were caused by the migrating flukes. It is, therefore, evident from Figure 1 that the migratory phase lasted for about six weeks and was followed by return of AST activity to normal range within the expected 21 days (Mullen, 1976).

GGT activity in the infected calves started to increase during the latter part of the migratory phase and became significantly higher than that in the controls during the patent stage of the infection (Figure 2). Comparison of increases in GGT and other enzymes including AST following necrosis of hepatocytes has shown that GGT activity remains unchanged or increases only slightly (Ideo *et al*., 1971; Ford 1974; Anderson *et al*., 1981). Most workers agree that GGT is a membrane-bound liver enzyme (Schlaeger et al; 1982; Halsall and Peters, 1984) and its location in the liver is mostly in the bile ducts (Naftalin *et al*; 1969; Mullen, 1976). These facts support the finding in the present study of higher values of GGT during the patent stage than prepatent stage of the infection. Its release from damaged bile ducts by the flukes is suggested to be enhanced by bile salts which solubilize membrane-bound enzymes (Schlaeger *et al*., 1982).

It is evident from Figure 3that the rate of growth of the infected animals declined at the time when AST and GGT activities were increasing. Decline in body weight gains in cattle infected with *F. gigantica* as well as *F. hepatica* has also been reported by other workers (Hammond and Sewell, 1974; Balbo *et al.,* 1973). However information about its relation to enzyme activities is lacking. As shown in Figure 3 the flattened portion of the growth curve indicating retardation in the growth of infected calves lies between PlW 8 and 12. This period lies well within the migratory phase of the infection during when there is steady increases in AST activity (Figure 1). This finding shows that liver parenchymal damage indicated by the rise of AST activity could be responsible for the retardation in growth. The major factor behind parenchymal liver damage which contributes to poor weight gains could be under utilisation of metabolites by the liver as observed in sheep infected with *F hepatica* (Sykes *et al*., 1980).

In conclusion, the observations in the present study on the serial patterns of AST and GGT emphasise the usefulness of these enzymes in earlier identification of the infection than the method of detecting eggs in the faeces. The results also shown that it is possible from the patterns to estimate the length of the migratory phase of the infection and the time when the flukes are in the bile ducts. As the poor weight gains evidently fell within the period when AST was on the increase, a combined serial body weight and parenchymal liver-specific enzyme determination could be of assistance in confirming/diagnosing the migratory phase of fasciolosis. Further extensive studies on liver specific enzyme activities and on different aspects of performance in infected animals are recommended in order to give more light on their usefulness in evaluating the relationship between liver damage and performance.

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