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The relationship between haematological indices, serum gamma-glutamyl transferase and glutamate dehydrogenase, visual hepatic damage and worm burden in cattle infected with *Fasciola gigantica*

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Abstract

The association between visual hepatic damage, burden of *Fasciola gigantica*, serum levels of gamma glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH) is described from an abattoir study of 70 cattle in the Philippines. In another abattoir study of 60 cattle, the relationship between burden of *F. gigantica* and haematological indices was investigated. The degree of visual hepatic damage and burden of *F. gigantica* were significantly positively related to levels of GGT and GLDH. Red blood cell counts and packed cell volume were significantly inversely related to worm burden, but animals compensated for reduced numbers of red blood cells by increasing red cell haemoglobin content.

Introduction

Infection with *Fasciola* spp. results in the liberation of hepatic enzymes into the blood and anaemia (Behm & Sangster, 1999). Elevated levels of gamma glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH) have been described in sheep (El Samani *et al.*, 1985; Roberts *et al.*, 1997) and in Indonesian cattle (Suhardono *et al.*, 1991) infected with *F. gigantica* and in water buffalo infected with *F. hepatica* (Yang *et al.*, 1998). Anaemia is the single most important factor contributing to the disease caused by liver fluke infections (Behm & Sangster, 1999). Anaemia has been reported in cattle and buffaloes infected with *F. gigantica* (Haroun & Hussein, 1976; Ogunrinade & Bamgboye, 1980; Taimur *et al.*, 1993; Yadav *et al.*, 1999) and in infections caused by *F. hepatica* (Behm & Sangster, 1999).

Knowledge of the association between clinical measurements that can be estimated in living animals and data on pathological changes in the liver as well as worm burden, which are only available post-mortem, would assist with the determination of the importance of the infection and the need to implement control. In addition, estimation of these enzymes provides an indication of the progression of infection as GLDH is elevated by damage to hepatic parenchyma by immature flukes, whereas GGT indicates damage to bile ducts due to the establishment of mature flukes (Sykes *et al.*, 1980; Galtier *et al.*, 1986; Ferre *et al.*, 1997).

The present study was designed to associate clinical measurements and pathological changes and to provide information from infection in cattle that may be used as diagnostic tools for the detection of bovine subclinical fascioliasis and possibly for the estimation of production losses due to the infection.

Materials and methods

Blood samples were collected by jugular venipuncture in Vacutainers containing ethylenediamine tetra-acetic acid (EDTA) as anticoagulant and in plain Vacutainers (Beckton Dickinson, Franklin Lakes, New Jersey, USA) from 70 cattle slaughtered at Davao abattoir and 60 cattle from Kabacan abattoir in Southern Mindanao, The Philippines.

The degree of gross hepatic damage was assessed qualitatively into one of the following categories: (i) worm-free; (ii) no visible lesions (NVL) but some worms

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Table 1. Mean values ± SD of gamma glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH) and number of *Fasciola gigantica* flukes in livers classified as free, no visible lesion (NVL), minor, moderate and severe ($n = 70$).

Category of hepatic pathology	GLDH ($\mu g\,l^{-1}$)	GGT ($U\,l^{-1}$)	Number of worms
Worm-free	5.13 ± 3.42	17.56 ± 8.16	0
NVL	12.16 ± 8.58	16.00 ± 2.45	5 ± 2.95
Minor	12.84 ± 6.89	21.79 ± 6.70	45 ± 25.84
Moderate	33.02 ± 15.13	27.38 ± 4.11	128.15 ± 23.36
Severe	59.22 ± 33.79	37.18 ± 5.02	353.0 ± 75.77

present; (iii) minor damage, few white fibrous foci present in the parenchyma; (iv) moderate damage, more numerous white fibrous lesions in the parenchyma; and (v) severe damage when the liver was enlarged, pale and fibrous.

Recovery of worms was done using the technique of Sinclair (1962). Worm burden was determined by counting the number of immature and mature intact individuals and worm heads. Worm burdens were categorized as no worms, 1–24 worms, 25–99 worms and ≥100 worms.

Levels of serum GGT and GLDH were determined using GGT and GLDH measurement kits (Boeringer Mannheim) from cattle slaughtered at Davao abattoir.

Haematological data were obtained from cattle slaughtered at Kabacan abattoir. Red blood cell (RBC) counts were carried out using a Neubauer hemocytometer. Packed cell volume (PCV) was determined by the microhaematocrit method by centrifugation at 10,000–12,000 rpm for 3–5 min. Haemoglobin values were determined using the acid-haematin method (Coles, 1986). Mean corpuscular haemoglobin (MCH) was estimated by dividing the haemoglobin present in grams per 1000 ml of blood by the total RBC count in millions per μl. Mean corpuscular volume (MCV) was obtained by dividing the PCV by the total RBC count in millions per μl. Statistical differences between groups were estimated using analysis of variance and a Tukey honestly significant difference (Tukey HSD) test using SPSS version 11.

Results

Increasing severity of hepatic changes due to infection with *F. gigantica* was generally associated with an increase in the number of worms recovered and with an increase in levels of GGT and GLDH (tables 1 and 2). There was a significant difference in values of GGT and GLDH between categories of hepatic pathology and worm burden.

A comparison of haematological values between animals grouped according to number of *F. gigantica* recovered is presented in table 3. Of the haematological values measured, erythrocyte counts were the most sensitive indicator of infection with counts negatively related to worm counts. Packed cell volume was also lower in animals with ≥100 worms than in those with fewer worms ($P = 0.07$). Mean corpuscular haemoglobin, on the other hand, increased significantly in animals with higher worm burdens.

Discussion

The present results are consistent with those of previous studies in sheep and Indonesian cattle infected with *F. gigantica* (El Samani *et al.*, 1985; Suhardono *et al.*, 1991; Roberts *et al.*, 1997) in that levels of GGT and GLDH increase during infection with *F. gigantica*. Levels of these enzymes were increased due to the presence of immature and mature flukes in the livers of the cattle, which caused hepatic parenchymal damage and bile duct epithelial damage, respectively. Significant increases in both GGT and GLDH were noted as hepatic damage and the level of infection increased. The present data therefore establish that serum levels of GGT and GLDH may be used as indicators of both levels of infection and severity of damage caused by *F. gigantica* and as indicators of associated physiological changes caused by subclinical fascioliasis in cattle.

The observation that PCV is reduced during infection with *F. gigantica* in cattle in the present study was also reported by Taimur *et al.* (1993) in cattle infected with *F. gigantica* who also found no difference in MCV between infected and uninfected animals. However, MCH values were not affected in their study, whereas, in the present study, MCH values were significantly higher in infected than in uninfected cattle, indicating that infected animals

Table 2. Multiple comparisons of mean glutamate dehydrogenase (GLDH) (μl^{-1}) and mean gamma glutamyl transferase (GGT) ($U\,l^{-1}$) values between pairs of categories of hepatic pathology. Only those comparisons giving a P value less than or a little more than 0.05 are shown ($n = 70$).

Category of hepatic pathology of groups being compared	Difference between mean values of GLDH between groups being compared	Difference between mean values of GGT between groups being compared
Worm-free and moderate	27.9 ($P = 0.00$)	9.8 ($P = 0.00$)
Worm-free and severe	54.1 ($P = 0.00$)	19.6 ($P = 0.00$)
NVL and moderate	20.0 ($P = 0.14$)	11.4 ($P = 0.02$)
NVL and severe	47.1 ($P = 0.00$)	21.1 ($P = 0.00$)
Minor and moderate	20.2 ($P = 0.00$)	5.6 ($P = 0.00$)
Minor and severe	46.4 ($P = 0.00$)	15.4 ($P = 0.00$)
Moderate and severe	26.2 ($P = 0.00$)	9.8 ($P = 0.00$)

Worm-free, $n = 18$; minor, $n = 24$; moderate, $n = 13$; severe, $n = 11$; no visible lesion (NVL), $n = 4$.

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Table 3. Multiple comparisons of mean blood values between cattle grouped according to level of infection with *Fasciola gigantica*. Only those comparisons with a *P* value less than or a little more than 0.05 are shown ($n = 60$).

Worm burden of groups being compared	Difference between mean values of RBC ($\times 10^6$ μl)	Difference between mean values of variable of PCV (%)	Difference between mean values of variable of MCH (pg)
0 and 25–99	1.9 ($P = 0.01$)		
0 and 100	3.0 ($P = 0.00$)		−19.2 ($P = 0.04$)
1–24 and 25–99	1.4 ($P = 0.07$)		
1–24 and 100	2.3 ($P = 0.00$)		−15.1 ($P = 0.04$)
25–99 and 100		6.0 ($P = 0.07$)	

MCH, mean corpuscular haemoglobin; PCV, packed cell volume; RBC, red blood cell.

in the present study compensated to some extent for reduced numbers of red blood cells by increasing the red cell haemoglobin content. Sewell *et al.* (1968) suggested that chronic fascioliasis causes stress on the haematopoeitic system, and it is possible that animals with light worm burdens may not show significant alterations in either haemoglobin or RBC values. The present results show that significant alterations were seen only in RBC and MCH values in infected cattle.

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