

THE INFLUENCE OF RABBIT INFECTION WITH INTESTINAL COCCIDIA UPON THE ACTIVITY OF LIVER ENZYMES

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Two groups of 10 rabbits each were infected with different doses of sporulated intestinal coccidia oocysts. Oocysts of the following intestinal coccidia were used as the infectious material: *Eimeria flavescens* (7%), *Eimeria matsubayashii* (9%), *Eimeria magna* (21%), *Eimeria neoleporis* (19%), *Eimeria perforans* (21%) i *Eimeria media* (32%). The third group (10 animals) served as a control. The rabbits acquired a subclinical form of coccidiosis which manifested itself in polydipsia, bristling hairs and weight loss, whereas only three rabbits developed full-blown illness with diarrhoea.

Immediately before the infection, then on the fourth, seventh, and tenth day following the infection with intestinal coccidia oocysts, the activity of several plasma enzymes was measured: aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and hydroxybutyrate dehydrogenase. Changes in the activity of those enzymes did not absolutely correlate with the numbers of inoculated infectious intestinal coccidia oocysts.

Key words: coccidiosis, rabbit, enzyme, liver

INTRODUCTION

Among the parasitic diseases of rabbits, coccidiosis is the most important infection since it is relatively frequent and causes major economic losses in rabbit-breeding. It is not easy to identify a rabbit free from various coccidia oocysts (Catchpole and Norton 1979, Polozowski 1993). Rabbit coccidiosis appears as a liver or intestinal form. The causative agents of rabbit coccidiosis have been studied by many investigators who focussed their attention primarily on the morphology of coccidia oocysts (Levin and Ivens 1972, Catchpole and Norton 1979). Very few authors published work pursuing changes in biochemical parameters in blood, urine and stools of rabbits with coccidiosis.

An alteration in the activity of alkaline phosphatase and amylase in the blood of sheep, rabbits and poultry with coccidiosis was registered by Catchpole and Gregory (1985), Šerkov *et al.* (1986), and Fukata *et al.* (1995).

In rabbits infected with *Eimeria intestinalis*, the activity of SGOT reached 26 IU/L in ten days following the infection as compared with 17.4 IU/L before the infection (Peeters *et al.* 1984). The activity of SGOT increased also on day 13 after the infection with intestinal coccidia but it was a fivefold increase (Šerkov *et al.* 1986).

Recent studies on intestinal coccidiosis of rabbits have dealt mostly with immunological aspects of the disease (Coudert *et al.* 1993, Licois *et al.* 1995).

The objective of this controlled study was to examine the course of activity changes of several enzymes in the blood of rabbits purposely infected with intestinal coccidia oocysts. The activity of the following enzymes was measured: aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and hydroxybutyrate dehydrogenase (HBDH).

MATERIALS AND METHODS

Male rabbits (Big Chincilla breed) aged 52 days and weighing 1200 - 1300 g were used in the study. Coprologic findings were consistently negative in all selected rabbits. All the rabbits had similar activity levels of AST, ALT, LDH, and HBDH. In order to determine the agents of rabbit intestinal coccidiosis we examined stool specimens of 410 rabbits from private breeders in Pančevo. The stool specimens were prepared for examination by the flotation method using a saturated solution of sodium chloride. Sporulation of coccidia oocysts was induced by incubating the stool specimens with added 2% solution of potassium bichromate at 27°C for 5 days. The samples were observed under a light microscope (immersion, magnification 12 x 40). Coccidial oocysts were identified according to the following morphological criteria: length, width, shape, color, wall width, and the presence of oocyst residual bodies. After the stool samples had been incubated in a thermostat at 27°C for 5 days, they were mixed with distilled water, centrifuged at 1500 rpm for 5 min, and the supernatant was discarded. The resulting pellet contained concentrated intestinal coccidia oocysts. The oocysts were counted in the McMaster chamber. The infectious dose of the oocysts was determined on the basis of the number of oocysts in 1 mL of the pellet. Oocysts of the following intestinal coccidia were used as the infectious material: *Eimeria flavescens* (7%), *Eimeria matsubayashii* (9%), *Eimeria magna* (21%), *Eimeria neoleporis* (19%), *Eimeria perforans* (21%) and *Eimeria media* (32%).

After an adaptation period of 10 days, rabbits were transferred to metabolic cages and divided into three groups of 10 animals each. The first group served as the control (0) - noninfected rabbits. Rabbits of the second and third groups were infected by direct instillation of oocysts through a tube into the empty stomach. Rabbits of the second group (A) were infected with 2×10^5 sporulated coccidia oocysts which were given in 100 mL of water. Rabbits of the third group (B) were infected with 4×10^5 infectious oocysts dispersed in 200 mL of the

infectious material, and given in two divided doses. Immediately before the infection, then on the fourth, seventh, and tenth, day following the infection with intestinal coccidia oocysts, blood samples were drawn and activities of the aforementioned enzymes were measured. Enzyme activities were determined on a spectrophotometer (Breckman) by the Warburg optical test (measurement of NADH concentration at 340 nm). The obtained results were statistically analyzed by analysis of the variance) Student's - test for a small sample).

RESULTS

Only three rabbits developed a complete clinical presentation of coccidiosis. The rabbits presented with diarrhoea, bristling hair, polydipsia, and weight loss. Other rabbits displayed milder signs of coccidiosis such as polydipsia, bristling hair and moderate weight loss. On day 10, coprological examination confirmed the presence of intestinal coccidia oocysts in the stools of all the infected rabbits.

It was obvious that the use of sporulated forms of coccidia oocysts was not sufficient to induce a full-blown clinical presentation of the disease but instead led to subclinical coccidiosis.

Aspartate aminotransferase activity. - The activity of AST in rabbits infected with intestinal coccidia apparently increased. The rise was more pronounced in the group of animals receiving the higher dose of the parasites (Figure 1). On the seventh day post infection, a significant increase of AST activity was detected in the group which had increased further on day 10. In contrast, in the group which

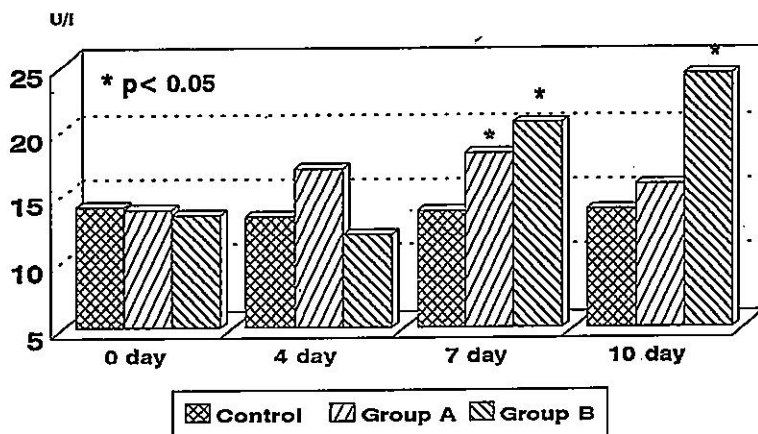


Figure 1. A comparative depiction of AST activity in rabbits following infection with intestinal coccidia.

received the lower dose, AST activity on day 10 was lower. Although significant, the rise in AST activity was relatively low.

Alanine aminotransferase activity - The activity of ALT in rabbits infected with intestinal coccidia was slightly elevated in both observed groups (Figure 2). The highest and significant increase in group (A) which received the lower dose of

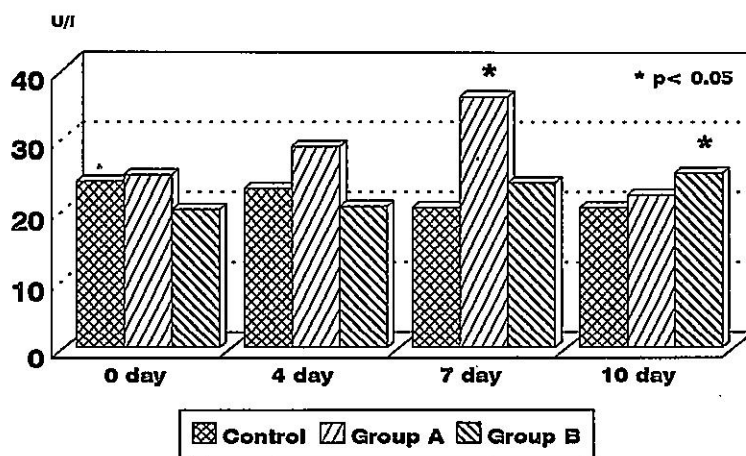


Figure 2. A comparative depiction of ALT activity in rabbits following infection with intestinal coccidia.

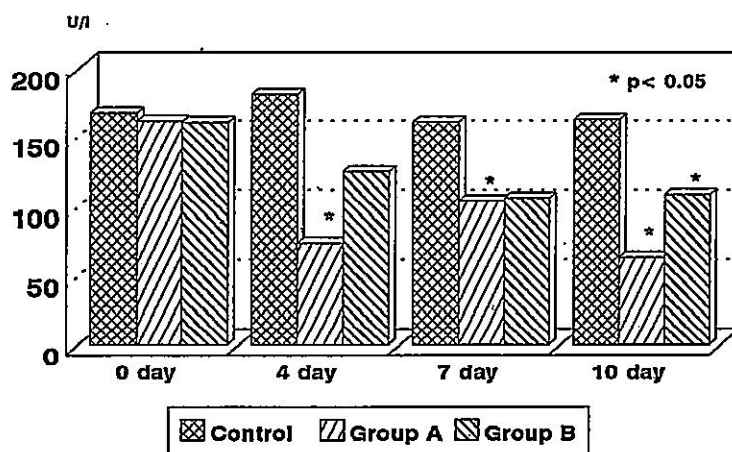


Figure 3. A comparative depiction of LDH activity in rabbits following infection with intestinal coccidia.

oocysts was registered on day 7 following the infection but by day 10 the values had returned to normal. In group B, the maximal increase was detected on day 10 after the infection. In the group variability of ALT activity was significant on day 7 post infection. Changes in the ALT activity in rabbits with coccidiosis correlated with corresponding changes in AST activity, and generally were mild.

Lactate dehydrogenase activity - The measured values of LDH activity in rabbits infected with intestinal coccidia showed a tendency towards a decrease in both infected groups (Figure 3). In rabbits infected with the lower number of oocysts, the decrease in LDH activity was even more pronounced. In all observation periods in both groups the decline in LDH activity was gradual reaching its peak on day 10. A significant inter group variability in LDH activity was observed on days 4 and 10 post infection.

Hydroxybutyrate dehydrogenase activity - In both groups of infected rabbits over the whole follow-up period, certain fluctuations in HBDH activity were detected with a clear trend towards declining (Figure 4). The decrease in HBDH activity followed the decrease in LDH activity. In that way, in rabbits infected with a lower dose of sporulated intestinal coccidia oocysts we detected a significant fall in HBDH activity at all time points with a maximum on day 10. A significant decrease in HBDH activity was observed on days 7 and 10 in the group of rabbits receiving a higher dose of the infectious material - at those time points the intergroup variability was also significant.

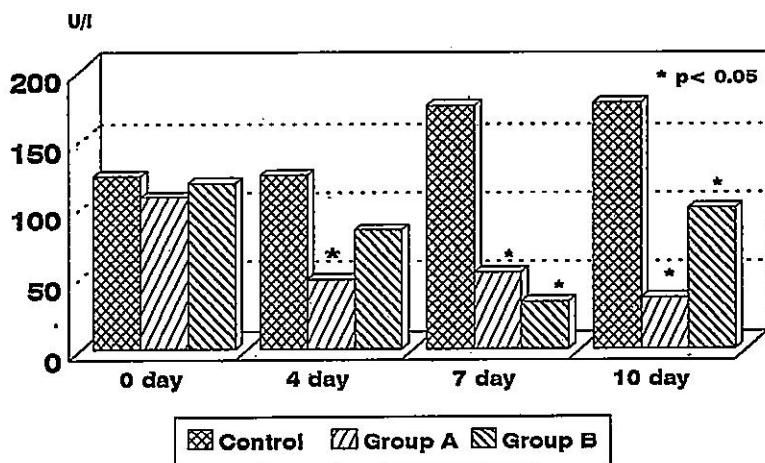


Figure 4. A comparative depiction of the HBDH activity in rabbits following infection with intestinal coccidia.

DISCUSSION

Over the observation period, an increase in AST activity was consistently detected. It was more pronounced in the group of rabbits given the higher dose of infectious oocysts. Inasmuch as the activity levels of this enzyme directly correlate with the damage to the liver tissue, the results suggest that the liver may be involved during coccidiosis. Possibly the products of coccidia might directly damage the hepatic tissue. On the other hand, disturbed physiologic processes in the intestinal lumen, epithelial desquamation, rupture of capillaries, and a subsequent disorder of intestinal absorption may lead to the additional hepatic damage due to the action of toxic intestinal products.

The works of Peeters et al (1984), and Šerkov et al (1986) showed an even greater increase in the activity of the enzyme in the course of intestinal coccidiosis suggesting similar pathogenetic mechanisms. Our results are in line with their findings.

Dynamics of ALT activity changes are closely correlated implying the same mechanisms operative in the upregulation of their activity.

Our findings showed that a decrease in the LDH activity occurred in both groups of rabbits with coccidiosis though it was less pronounced in the group given the lower dose of oocysts. Since in the course of intestinal coccidiosis the intestinal mucosa is severely damaged there is a decrease in nutrient absorption and their concomitant loss into the intestinal lumen. This lowers the input of the LDH substrate, glucose, the concentration of which is decreased in infected rabbits, again to a greater degree in the group receiving the higher number of oocysts. Hence less lactate is produced and available to LDH which converts it to pyruvate which may cause downregulation of LDH synthesis. Shortage of substrates is generally known to regulate negatively the synthesis of corresponding enzymes (Majkić-Singh 1993). As far as we are aware there are no published data on changes in LDH activity in rabbits suffering from coccidiosis.

As with LDH, the activity of HBDH is significantly decreased at all observation points in both rabbit groups. As HBDH represents an isozyme of LDH it closely follows the fluctuation in the activity of LDH.

Our results show that a subclinical form of rabbit coccidiosis, induced by different species of intestinal coccidia oocysts, leads to changes in the activity of liver enzymes - an elevation of AST and ALT activity, and a fall in the activity of LDH and HBDH.

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UTICAJ INFEKCIJE CREVNIM KOKCIDIJAMA KUNIĆA NA AKTIVNOST ENZIMA JETRE

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SADRŽAJ

Različitim oocistama crevnih kokcidija (*Eimeria flavescens*, *Eimeria mat-subayashii*, *Eimeria magna*, *Eimeria neoleporis*, *Eimeria perforans* i *Eimeria media*) izazvali smo eksperimentalnu kokcidiozu kod dve grupe po 10 kunića. Prvu grupu smo inficirali sa 2×10^5 , a drugu sa 4×10^5 infektivnih oocista. Kunići su oboleli od subkliničke kokcidioze. Neposredno pre infekcije, zatim četvrtog, sedmog i desetog dana, određivali smo u krvi aktivnost sledećih enzima: aspartat aminotransferaze (AST), alanin aminotransferaze (ALT), laktat dehidrogenaze (LDH) i hidroksibutirat dehidrogenaze (HBDH). U plazmi smo registrovali povećanje aktivnosti aspartat aminotransferaze i alanin aminotransferaze, te smanjenje aktivnosti laktat dehidrogenaze i hidroksibutirat dehidrogenaze. Promena aktivnosti posmatranih enzima nije bila apsolutno saglasna sa brojem inokulisanih sporuliranih oocista crevnih kokcidija.