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Eimeria stiedai: metabolism of lipids, proteins and glucose in experimentally infected rabbits, *Oryctolagus cuniculus*

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Abstract

Rabbits were experimentally infected with sporulated *Eimeria stiedai* oocysts. A total of 50 white adult rabbits, New Zealand race, were distributed into two groups: group A was infected with $1x10^4$ sporulated *Eimeria stiedai* oocysts, while group B was inoculated with distilled water as a control. The animals generally displayed increased levels of total protein, globulin, total cholesterol, LDL-c and triacylglycerols; however, total levels of liver lipids and HDL-c decreased, and plasma glucose levels varied during the experimental period. In sum, *Eimeria stiedai* infection of rabbits caused a considerable number of changes in the metabolism of lipids, proteins and glucose, which is likely due to direct effects of liver cirrhosis on normal body function.

Key Words: *Eimeria stiedai,* Apicomplexa, metabolism, rabbits.

Introduction

The rabbit meat production industry has grown considerably in the past few years, raising concerns of pathogen control to reduce economic losses and increase profits. Among the various diseases that afflict rabbits, we highlight eimeriosis because this disease creates serious health problems due to various changes in general metabolism (11). Domestic rabbits can be infected by up to eleven species of *Eimeria* genera including *E. stiedai*, which is known to parasitize the liver and cause hepatic coccidiosis. *E. stiedai* generates schizonts and forms oocysts in the bile ducts (5,18). These oocysts are subsequently eliminated through the intestine (via the common bile duct) and exit the organism through the feces and contaminate the environment.

The parasite prepatent period lasts approximately 15 days, while the patent period is usually 35 days (3), which represents a significant period of time since rabbits are normally slaughtered between 70 to 80 days of age. Several authors have reported various physiological and pathological effects caused by coccids on different hosts, and lesions were found in different parts of the body depending on the type of *Eimeria* species infected (6, 12, 14,10). Thus, animals infected by *E. stiedai* are underdeveloped and slaughtered at lower than optimal body weights, causing substantial economic losses in this industry.

The liver performs various vital functions, including regulation of metabolism, protein synthesis, iron and vitamin storage, hormone degradation as well as inactivation and excretion of drugs and toxins from the body. The liver also stores glycogen, which is then degraded into glucose to maintain normal blood glucose concentrations. Lipids represent the largest energy sources in the body and are intimately involved in the structure of membranes and formation of essential steroid hormones, prostaglandins and bile acids (7). Apolipoproteins are composed of lipids and proteins and can be divided into five major classes differentiated by size, density and by lipid and apoprotein composition: chylomicrons (Qm), very low-density lipoproteins (VLDL-c), intermediate density lipoproteins (HIL-c), low-density lipoproteins (LDL-c) and high-density lipoproteins (HDL-c) (8).

As only a few studies describe the mechanism of damage caused by *E. stiedai* infection, details about

the post-infection period and the impact of the infection on the host animal's health currently remain unknown. Thus, further studies are needed to characterize *E. stiedai* pathophysiology (11). Here we aimed to evaluate changes in the metabolism of lipids, proteins and glucose in rabbits experimentally infected with *E. stiedai* oocysts.

Materials and Methods

This research was conducted in the Department of Veterinary Pathology, Faculty of Agriculture and Veterinary Sciences at Universidade Estadual Paulista (UNESP-Jaboticabal), Jaboticabal-SP.

Two white New Zealand adult male rabbits were immunosuppressed by intramuscular administration of dexamethasone to obtain oocysts. After immunosuppression, the animals were infected orally with $1x10^5$ sporulated *Eimeria stiedai* oocysts. Twelve days after oocyst inoculation (dpi), the animals were sacrificed, and oocysts were obtained by puncturing the gallbladder (Figure I). Oocysts were allowed to sporulate for a period of 48 hours in 2.5% potassium dichromate under environmental temperature and humidity.

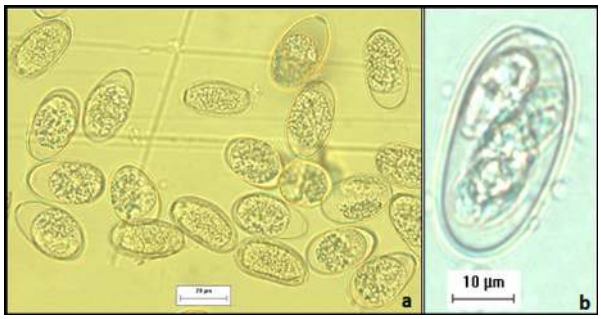

Figure 1 - Material obtained from oocysts donor animals at 12° dpi. a) Non-sporulated *Eimeria stiedai* oocysts obtained after puncture of donor animals hepatic gallbladder. b) Sporulated *Eimeria stiedai* oocysts used in the infection of experimental animals.

Experiments were conducted using 50 white, New Zealand rabbits of similar weight between 40-50 days of age. The animals were evenly and randomly divided into two groups (group A-Infected and group B-control) and placed in separate locations under similar environmental conditions. Each group of animals was provided with clean water and balanced food free from anticoccidial drugs. The feeders and drinkers were thoroughly washed with detergent and flamed every 24 hours to avoid risk of re-infection. All experimental animals were manually restrained and inoculated with the aid of an intragastric probe. Animals belonging to group A were inoculated with 1.0 mL of $1x10^4$ sporulated *E. stiedai* oocysts, while group B was inoculated with 1.0 mL of distilled water.

Blood samples were taken at 0, 7, 14, 21 and 28 dpi by cardiac puncture using sterile needles and syringes. Blood tubes were then sent to the Clinical Veterinary Surgery Department at the Faculty of Agricultural Sciences and Veterinary Jaboticabal/UNESP for biochemical analysis. The plasma glucose levels were determined using a enzymatic commercial kit (Labtest Diagnostica, Belo Horizonte, MG) and read immediately after collection in a spectrophotometer (LabQuest Diagnostica, Belo Horizonte, MG). The following seric lipids were measured in the blood serum: lipoprotein and triacylglycerols were quantified using the previously described method by Nagele et al. (17); total cholesterol was measured using the method proposed by Alain et al. (1) and modified by Good et al. (13); and HDL-c. All samples were measured using a commercial kit (Labtest Diagnostica, Belo Horizonte, MG) and read in a spectrophotometer (LabQuest Diagnostica, Belo Horizonte, MG). LDL-c levels were calculated using the following formula: LDL-c = total cholesterol – HDL-c – VLDL-c. Total hepatic lipid concentrations at 7, 14, 21 and 28 dpi were determined gravimetrically after extraction with chloroform:methanol (2:1, v/v) as described by Bligh and Dyer (4).

The animals were sacrificed by cervical dislocation following guidelines from the Commission on Ethics, Bioethics and Animal Welfare (CEBEA) of Jaboticabal / UNESP. The data were evaluated using Wilcoxon tests at a significance level of 5%.

Results and Discussion

Table I presents laboratory test data. Total protein and globulin concentrations increased significantly in 28 dpi animals, while serum albumin levels of infected animals remained within normal limits throughout the experimental period. The observed decrease in serum albumin levels may be related to hepatic injury, as albumin is produced exclusively in the liver (16). However, total protein and albumin can be found at normal levels even in the presence of hepatic injury, unless the injury to the liver is major and the hepatitis is severe enough to cause liver failure (19). According to our results, however, we observed normal serum albumin concentration during the hepatic inflammatory process caused by parasite reproduction.

Infected animals presented hypoglycemia at the end of the experimental period, possibly from mobilization of organic reserves to replace deficits in hepatic glycogen stores due to infection. Total cholesterol, triglycerides and LDL-c increased significantly in infected animals after 14 dpi and remained above normal concentrations until 28 dpi. The total liver lipid and HDL-c obtained were below normal concentrations beginning at 21 dpi, but HDL-c levels also showed a significant decrease in the control group starting from 7 dpi.

Table I- Mean values of lipids, glucose e total protein in the serum of rabbits experimentally infected with *Eimeria stiedai*.

Parameters	Days after infection				
	0	7	14	21	28
Total protein					
Control	6,83^{ns} (6,18-7,91)	7,19^{ns} (6,83-7,58)	7,33^{ns} (6,75-7,77)	7,37^{ns} (6,64-8,28)	6,75^{b} (6,29-7,56)
Infected	6,75^{ns} (6,29-7,27)	6,75^{ns} (6,29-7,38)	7,23^{ns} (6,03-8,08)	6,68^{ns} (5,98-7,47)	9,50^{a} (8,95-9,30)
Albumin					
Control	3,54^{ns} (3,05-4,18)	3,75^{ns} (3,19 -4,22)	3,76^{ns} (3,05-4,39)	3,42^{ns} (3,15-3,77)	3,28^{ns} (3,09-3,57)
Infected	3,51^{ns} (3,02-4,22)	3,34^{ns} (3,07-3,60)	3,62^{ns} (3,42-3,84)	3,38^{ns} (3,04-3,82)	3,63^{ns} (3,28-3,96)
Globulin					
Control	3,29^{ns} (2,56-3,92)	3,44^{ns} (2,98-3,78)	3,57^{ns} (3,03-4,15)	3,94^{ns} (3,24-4,88)	3,48^{b} (2,97-4,35)
Infected	3,24^{ns} (3,03-3,95)	3,42^{ns} (3,03-3,94)	3,60^{ns} (2,19-4,50)	3,29^{ns} (2,88-3,49)	5,87^{a} (5,10-6,93)
Total Cholesterol					
Control	92,02^{ns} (57,70-111,10)	95,01^{a} (81,10-111,70)	80,01^{b} (64,86-103,90)	73,74^{b} (42,10-190,10)	60,32^{b} (36,60-76,30)
Infected	98,02^{ns} (92,50-103,90)	63,04^{b} (41,11-76,62)	121,30^{a} (106,50-160,70)	157,08^{a} (115,20-201,20)	200,08^{a} (87,22-388,70)
LDL-c					
Control	66,10^{ns} (29,40-87,50)	64,29^{a} (40,50-76,80)	46,61^{b} (33,86-68,30)	49,44^{b} (33,80-86,50)	42,66^{b} (24,00-52,40)
Infected	71,22^{ns} (66,80-74,40)	41,22^{b} (21,81-58,00)	103,92^{a} (91,90-133,80)	144,86^{a} (95,10-193,70)	191,40^{a} (77,62-376,50)
HDL-c					
Control	25,29^{ns} (17,20-39,10)	30,72^{ns} (23,00-40,60)	33,40^{a} (21,40-41,80)	24,30^{a} (20,70-28,00)	17,66^{a} (11,50-24,10)
Infected	26,80^{ns} (21,40-35,60)	21,82^{ns} (10,90-30,70)	17,38^{b} (13,40-26,90)	12,22^{b} (5,00-20,10)	8,68^{b} (5,80-12,20)
Total hepatic lipids (%)					
Control	-	2,29^{ns} (1,933-2,722)	1,79^{b} (1,218-2,244)	2,20^{a} (1,609-2,876)	2,72^{a} (1,749-3,357)
Infected	-	2,26^{ns} (1,85-2,508)	2,01^{a} (1,357-2,744)	1,54^{b} (1,320-1,815)	1,09^{b} (0,5082-1,380)
Triacylglycerols					
Control	159,26^{ns} (107,50-263,50)	160,42^{ns} (98,20-233,50)	103,06^{b} (85,20-123,60)	174,06^{ns} (91,30-234,60)	142,30^{b} (113,20-202,30)
Infected	141,74^{ns} (107,50-215,09)	128,12^{ns} (92,00-193,10)	248,06^{a} (215,80-303,30)	338,02^{ns} (142,00-58,30)	456,86^{a} (268,00-644,10)
Plasmatic Glucose					
Control	111,00^{ns} (104,00 – 121,00)	103,80^{a} (98,00-110,00)	95,80^{b} (85,00– 110,00)	94,60^{b} (86,00 -101,00)	98,60^{a} (91,00 – 110,00)
Infected	109,20^{ns} (94,00– 121,00)	94,60^{b} (80,00 – 109,00)	106,80^{a} (98,00– 315,1)	102,80^{a} (89,00 –110,00)	81,60^{b} (80,00 – 90,00)

n.s: not significant by the Wilcoxon test at 5% probability.
Means followed by the same letter do not differ statistically.

Here we show that parasite reproduction caused a reduction in the expression of LDL-c receptors in hepatocytes, contributing to increases in LDL-c serum concentration in infected animals. The increase in total serum cholesterol was likely due to a deficiency of Apolipoprotein AI (Apo-AI), which is needed for HDL-c production. This protein is synthesized mainly in the liver, which was damaged in our experiments. Low HDL-c levels were shown to hinder the reverse cholesterol transport pathway. Humans with Apo-AI deficiency or mutation, complete or partial lecithin-cholesterol acyltransferase (LCAT) deficiency, and ABCAI transport-related deficiencies were also shown to disrupt this pathway. Low HDL levels in humans are commonly found to be associated with smoking (due to lower LCAT levels), visceral obesity (by reducing LCAT and LLP), very low-fat diets, hypertriglyceridemia, and the use of certain drugs (2). Parasite-induced infection by *Dicrocoelium dentricum* (20), *Fasciola hepatica* (15), and *E. stiedai* (5,11) cause destruction of hepatic parenchyma and bile ducts, resulting in lipid peroxidation due to the presence of free radicals derived from the inflammatory process.

The few studies describing lipid metabolism in rabbits experimentally infected with *E. stiedai* are restricted to only those changes in hepatic glycogen levels and total cholesterol. Previously, while searching for metabolic changes in chickens experimentally infected with the enteric parasite *E. acervulina*, observed that the levels of all classes of serum lipids and lipoproteins were reduced in infected animals (9).

Furthermore, this reduction in serum lipids was associated with a significant increase in hepatic fat. While assessing pathological changes in rabbits under the same experimental conditions of this work, observed ascites, hepatomegaly, cirrhosis and liver fibrosis as well as thickening of the gallbladder wall, all of which resulted in considerable changes to hepatic enzyme concentrations (11).

Conclusion

Infection by the *E. stiedai* parasite leads to significant changes in the metabolism of lipids, proteins and glucose, which is likely due to the direct effect of cirrhosis on normal liver function.

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