

Short Communication

Prevalence of rabbit hepatic coccidiosis in north west of Iran

Tehrani¹, A.A., Yakhchali^{*1}, M., Beikzadeh², B., Morvaridi³, A.

1. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Graduated in Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
3. Graduated student of M.Sc. of Immunology, Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Received 14 Aug 2012; accepted 26 Nov 2012

ABSTRACT

Hepatic coccidiosis is considered as a major problem in rabbits which mortality rate may go high as a result of unhygienic maintenance with overcrowding. This study was aimed to determine abundance and pathologic changes of hepatic coccidiosis in rabbits of northwestern Iran. A total of 320 rabbits (110 New Zealand, 110 Angora, and 100 Native) in different sex and age groups were randomly selected from rabbitries in northwestern Iran. The rabbits were kept either individually in cages or in groups in floor pens. They reproduced for research and instruction. Fecal samples were collected from cages and floor pens and subjected to flotation techniques. The collected liver tissues fixed in 10% buffered formal saline, sectioned, and stained with Hematoxyline and Eosin (H&E). Results indicated that infection rate with *E. stiedae* was 26.87% (86/320). The prevalence of *E. stiedae* was significantly higher in weanling rabbits (5-8 months) (9.69%, 31/320) than other age groups. There was no significant difference in the prevalence of *E. stiedae* between male and female rabbits. At necropsy, numerous and scattered white nodules about 0.1 to 0.5 cm in diameter were observed on the liver surface. Histopathological lesions included hyperplasia of the bile duct epithelium with different developmental stages of coccidian agents. Granuloma tissues encircle the bile duct with infiltration of inflammatory cells. It was concluded that hepatic coccidiosis was common in Iranian rabbits of the region and with proper management and strict hygiene and sanitation can effectively control the rate of infection in the rabbitries.

Keywords: Prevalence, *Eimeria stiedae*, Rabbit, Iran

INTRODUCTION

Rabbit coccidiosis is a common and widespread protozoal infection in commercial operations and is responsible for major pathogenicity in definitive host (Manning *et al* 1994, Cere' *et al* 1996, Al-Quraishy 2012). It is an important economic and disease of rabbits, especially, in breeding and rearing

establishments where sanitation measures are poor. Rabbit coccidiosis has two anatomic forms: hepatic caused by *E. stiedae* and intestinal caused by other fourteen *Eimeria* species (Li & Ooi 2009). Hepatic coccidiosis is most often subclinical, but growing rabbits may fail to make normal gains. With the exception of *E. magna*, the identification of species is difficult on oocyst alone (Al-Rukibat *et al* 2001). Often histopathological examination of lesions is therefore

*Author for correspondence. Email: m.yakhchali@urmia.ac.ir

required to rule out other causes of the disease. Based on this, the present study was aimed to have a preliminary estimation of prevalence of rabbit hepatic coccidiosis, associated predisposing factors (age, sex, and breeds), and pathologic changes of infected liver by *E. Stiedae* in North West Iran.

MATERIALS AND METHODS

Animals. A total of 320 rabbits in different age groups and breeds of New Zealand (n=110), Angora (n=110), and Native (n=100) breeds were randomly selected. All of them did not receive any anti coccidian treatment. The rabbits were kept either individually in cages or in groups in floor pens. All of them were domesticated rabbits and did not receive any anti coccidian treatment. The main diet of the rabbits consisted of commercially prepared pellets, although, they were fed with low amounts of alfalfa, lettuce, carrot, and apple to provide the required fiber. The main purpose of rabbit production in this region is to provide laboratory rabbits for instruction and research.

Parasitological procedures. Faecal samples were randomly collected from cages and floor pens and put into a plastic container with a lid. The data pertaining to the sex, age, and breeds were recorded. Fresh fecal samples were collected and performed to carry out flotation technique. To detect *Eimeria* oocysts, a part of each sample (3g) was mixed with tap water (42ml) and subjected to the flotation technique using Sheather solution (specific gravity 1.12) and centrifuged (2000 rpm for 3 min) (Hendrix 1998). Oocyst per gram of feces (OPG coefficient) was also measured by using modified McMaster technique (Anon 1984). Fecal samples of infected rabbits were processed individually using dichromate potassium (2.5% $K_2Cr_2O_7$, w/v) to allow oocysts to sporulate. Phenotypic characters of the oocysts including morphology and morphometry were used for identifying *E. Steidae* (Eckert et al 1995).

Histopathological examination. The remaining infected rabbits with *E. steidae* oocyst shedding were sedated by Ketamine and euthanized using Sodium

pentobarbital (100 mg/kg, IV) according to a method consistent with recommendation of panel on Euthanasia of the American Veterinary Medical Association. Infected livers were subjected for histological examination. The liver tissues were fixed with 10% neutral formal saline for paraffin embedding. The sectioned livers (5 μ m thick pieces) were stained with routine haematoxylin and eosin (H&E) and examined under light microscope.

Statistical evaluation. Chi-square test was used for analysing statistical association between the data findings by using SPSS statistical program (version 14, SPSS Inc., Chicago, IL, USA). Probability of <0.05 was regarded as significant with confidence interval of 95%.

RESULTS AND DISCUSSION

The sex, breeds, and age of infected rabbits were shown in Table 1. The results indicated that 26.87% (86/320) rabbits were infected by *E. stiedae*. Of those, 12.5% (40/320) and 14.37% (46/320) were male and female, respectively. The prevalence of *E. stiedae* was significantly higher in weanling rabbits (5-8 months) (9.69%, 31/320) than other age groups ($P<0.05$). In adult rabbits (>12 months), *E. stiedae* was low and may serve as carrier (3.75%, 12/320). There was no significant difference in the prevalence of *E. stiedae* between male and female rabbits ($P>0.05$).

At necropsy, macroscopically, the liver was enlarged and had multiple nodules of 2 to 4 mm in size. The parenchyma was congested and edematous (Figure 1). Bile ducts chronically inflamed and dilated with bile and exudates. Histological findings in the liver consisted epithelium hyperplasia of bile ducts, enlargement of bile ducts with infiltration of inflammatory cells (lymphocytes and plasmacells). Biliary cirrhosis was extensive in almost all the infected animals (Figure 2). The Lumina of the bile ducts were filled with numerous oocysts in the lumen with chronic proliferative cholangitis and cholecystitis (Figure 3). The present study reports the incidence and

Table 1. Prevalence of *Eimeria stiedae* infection according to sex, age, and breed of examined rabbits (n=320).

No. of examined animals	Prevalence (%)	Breed (%)			Age (Month, %)								Sex (%)									
		Nz	An	Na	1-4			5-8			9-12		>12			M			F			
					Nz	An	Na	Nz	An	Na	Nz	An	Na	Nz	An	Na	Nz	An	Na	Nz	An	Na
320	26.87	8.75	8.13	10	3.13	2.5	1.56	3.4	4.1	2.2	2.5	2.5	1.3	1.6	1.3	0.9	4.7	4.4	3.4	5	5.3	4.1
Total					7.19				9.69 ^S				6.25			3.75	12.5				14.37 ^{NS}	

An: Angora, F: female, M: male, Na: Native, NS: non-significant (χ^2 , $P > 0.05$), Nz: New Zealand, S: significant (χ^2 , $P < 0.05$)

pathological lesions one of the important coccidian parasite of rabbits, *E. stiedae* in northwestern Iran. The reported rate of infection was considered low compared with other studies in Saudi Arabia (32.4%) (Al-Mathal 2008). There were also several reports about hepatic coccidiosis with a high rate of infection all over the world (Gomez-Bautista *et al* 1987). In contrast, Zarzara *et al.* (1989) found that the coccidian infection of liver was nearly 0.1%. While in Syria the abundance of infection was 4% (Darwish & Golemansky 1991).

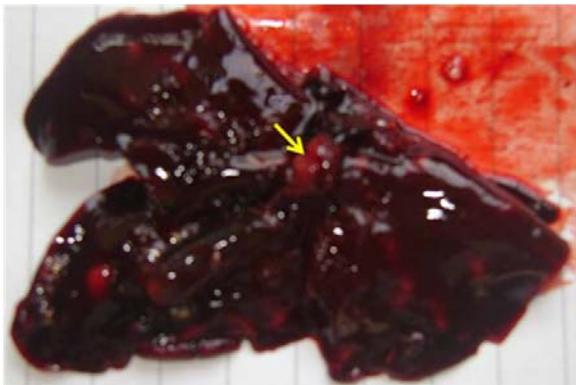


Figure 1. Focal nodular (arrow head) hyperplasia most recognizable gross feature in rabbit hepatic coccidiosis.

These variations may depend on geographical location, the difference in environmental conditions prevailing in each region, the rearing conditions, the number of samples examined, and the season of the year of the study (Al-Mathal 2008). In earlier studies by Catchpole and Norton (1979) and Peeters & Geeroms (1986), *E. stiedae* was not reported as a common protozoal infection in rabbits. While in the

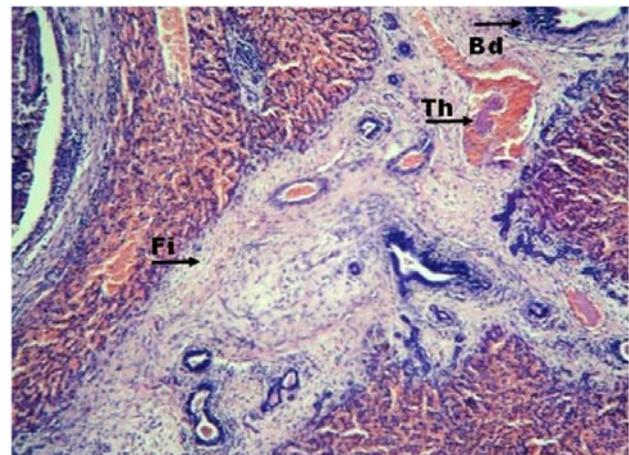


Figure 2. Biliary cirrhosis. Fibrous (Fi) tissue encircles the bile ducts (Bd). There is a thrombosis (Th) in the veins (H&E, 100×).

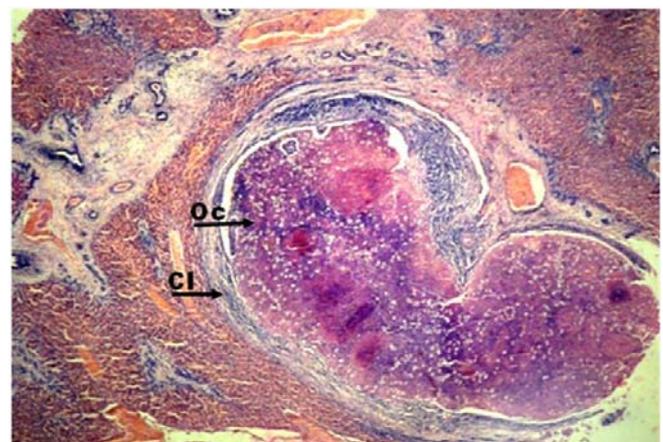


Figure 3. Cholangitis (Cl) with hypertrophied and hyperplastic bile duct. The bile duct is severely dilated and filled with oocysts of *E. stiedae* (Oc) (H&E, 100×).

present study findings indicated that it is an important cause of infection in Iranian rabbits was *E. stiedae*. Gres et al. (2003) reported the same finding in wild rabbits. The infection rate observed more in weanling rabbits in which there was no sex and breeds differences. This isolate of *E. stiedae* multiplies in the epithelial cells of bile ducts and is considered as a pathogenic organism (Hauptman et al 2001, Al-Mathal 2008). *E. stiedae* like other *Eimeria* species enters into the intestinal wall, but it then migrates to the bile ducts, where it reproduces (Kraus et al 1984). The hepatic coccidiosis caused severe damage to the liver, particularly, in young rabbits and led to death (Wang & Tasi 1991, Yakhchali & Tehrani 2007). The remarkable pathological characteristic in the liver of infected animals was a well biliary cirrhosis that presented a perilobular fibrosis similar to that of atrophic cirrhosis. However, careful observation revealed that the collagen fibers started to collect get organised around the bile ducts. Al-Mathal (2008) noted that in rabbit hepatic coccidiosis hyperplasia and enlargement of bile ducts with infiltration of inflammatory cells (eosinophiles, lymphocytes and plasma cells) were evident. Biliary outflow may be obstructed by oocysts, resulting in a distended bile duct. The liver parenchyma may be destroyed by the pressure from the expanding biliary ducts and gradually replaced by fibrous connective tissue (Erdogmus & Eroksuz 2006). From the results of the present study, it was concluded that rabbit hepatic infection due to *E. stiedae* was common in the region and proper management and good programmed husbandry are necessary to reduce the rate of infection.

Acknowledgements

Authors are grateful to K. Badali at Parasitology division for technical assistance and Mr. Farokhi for animal maintenance during the course of study.

References

- Al-Mathal, E.M. (2008). Hepatic coccidiosis of the domestic rabbit *Oryctolagus cuniculus domesticus* L. in Saudi Arabia. *World Journal of Zoology* 3(1):30-35.
- Al-Quraishy, S. (2012). Exogenous and endogenous stages of *Eimeria perforans* naturally infected domestic rabbit (*Oryctolagus cuniculus*) in Saudi Arabia: Light microscopic study. *Saudi Journal of Biological Sciences* 19:31–34.
- Al-Rukibat, R., Irizarry, K., Lacey, A.R., Kazacos, K., Storandt K.R. and DeNicola, S.T. (2001). Impression smears of liver tissue from a rabbit. *Veterinary Clinical Pathology* 30:57-61.
- Anon, A. (1984). *Manual of veterinary parasitological laboratory techniques*. Pp: 11–12, Ministry of Agriculture, Fisheries and Food. Majesty Stationary Office, London, UK.
- Catchpole, J. and Norton, C.C. (1979). The species of *Eimeria* in rabbits for meat production in Britain. *Parasitology* 79:249-57.
- Cere', N., Humbert, J.F., Licois, D., Corvione, M., Afanassieff, M. and Chanteloup, N. (1996). A new approach for the identification and the diagnosis of *Eimeria media* parasite of the rabbit. *Experimental Parasitology* 82:132–138.
- Darwish, A.I. and Golemansky, V. (1991). Coccidian parasites (Coccidia: Eimeriidae) of domestic rabbits (*Oryctolagus cuniculus domesticus* L.) in Syria. *Acta Parasitologica* 30:209–215.
- Eckert, J., Taylor, M., Licois, D., Coudret, P., Catchpole, J. and Bucklar, H. (1995). Identification of *Eimeria* and *Isospora* species and strains: Morphological and biological characteristics. In: Eckert J, Braun R, Shirley MW, et al. (eds) *Biotechnology. Guidelines on techniques in coccidiosis research office for official publications of the European communities, Luxembourg*, Pp: 103-119.
- Erdogmus, Z.S. and Eroksuz, Y. (2006). DS hepatic coccidiosis in Angora rabbits. *Journal of Animal and Veterinary Advances* 5(6): 462-463.
- Gomez-Bautista, M., Rojo-Vazquez, F.A., Alunda, J.M. (1987). The effect of the host's age on the pathology of *Eimeria stiedae* infection in rabbits. *Veterinary Parasitology* 24: 47-57.
- Gres, V., Voza, T., Chabaud, A. and Landau, I. (2003). Coccidiosis of the wild rabbit (*Oryctolagus cuniculus*) in France. *Parasite* 10:51-57.
- Hauptman, K., Tichy, F. and Knotek, Z. (2001). Clinical diagnostics of hepatoopathies in small mammals: Evaluation of importance of individual methods. *Acta Veterinaria Brno* 70: 297-311.
- Hendrix, C.M. (1998). *Diagnostic veterinary medicine* (2nd edn). Pp: 256-259, Mosby.

- Kraus, A.L., Weisbroth, S.H., Flatt, R.E. and Brewer, N. (1984). *Biology and diseases of rabbits*. In: laboratory animal medicine Fox, J.G. (ed.), Pp: 270-240, Academic Press, Orlando, USA.
- Li, M. and Ooi, H. (2009). Fecal occult blood manifestation of intestinal *Eimeria* spp. infection in rabbit. *Veterinary Parasitology* 161:327-329.
- Manning, P.J., Ringler, D.H. and Newcomer, C.E. (1994). *The Biology of the laboratory rabbit* (2nd edn.). Academic Press, San Diego, USA.
- Peeters, J.E. and Geeroms, R. (1986). Efficacy of toltrazuril against intestinal and hepatic coccidiosis in rabbits. *Veterinary Parasitology* 22:21-35.
- Wang, J.S. and Tasi, S.F. (1991). Prevalence and pathological study on rabbit hepatic coccidiosis in Taiwan. *Proceedings of the National Science Council, Republic of China. Part B, Life Sciences (Taipei)* 15(4):240-243.
- Yakhchali, M., Tehrani, A. (2007) Eimerioidosis and Pathological Findings in New Zealand White Rabbits. *Journal of Biological Sciences* 7: 1488-1491.
- Zarzara, C., Stanescu, V., Marcoci, M. and Popovici, A. (1989). Incidence of hepatic and intestinal coccidiosis in farmed rabbits. *Lucrarile Institutului de Cercetari Veterinare si Biopreparate Pasteur* 18:141-145.