

Pathology and Therapy in Naturally *Eimeria stiedae*-Infected Rabbits

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Key Words: *Eimeria stiedae*, Therapy, Pathology, rabbit

ABSTRACT

Clinical hepatic coccidiosis in New Zealand white rabbits (n=24) of either sex (aged 1-2 month) with clinical signs, pathology and therapy is presented. The infected rabbits showed anorexia, reluctance to move and death within 3-4 days. Liver showed irregular whitish nodules scattered on its surface and in deeper parenchyma. Characteristic histopathological changes in liver with different developmental stages of *Eimeria stiedae* in the epithelial cells of bile ducts have been recorded. Treatment of infected rabbits with 2.5% oltrazuril (Baycox) at 25 ppm in water for 2 days was highly effective. The oocysts appeared in the faeces on day 6 after treatment. No mortality was seen in treated rabbits while all the infected untreated rabbits died within a period of 7 days.

INTRODUCTION

Coccidiosis is considered to be a major problem in rabbits as mortality rate may go high particularly during and after rainy season (Gill and Ray, 1960). Adults which are usually symptomless carriers of coccidial infection serve as potential source of severe infection with clinical signs resulting in to death of younger ones (Bull, 1958, Wang and Tsai, 1991). Among thirteen *Eimeria* species infecting rabbits in different parts of the world, *E. stiedae* which parasitizes the epithelial cells of the bile duct is extremely pathogenic intracellular extra intestinal coccidial species (Hung et al., 1984). The need for evolving better compounds against coccidiosis has been felt due to the emergence of drug resistance. Toltrazuril is a coccidiocidal drug which is very effective against coccidiosis in rabbits, pigs, sheep and cattle (Haberkorn and Mundt, 1988). The first clinical trial on rabbit intestinal coccidiosis with toltrazuril indicated successful treatment with 1ml per litter (Zurliiski and Vladimirova, 1988). However, it has not previously been tried against rabbit coccidiosis in India. It affects all the intracellular

developmental stages (asexual and sexual) of coccidian parasite and does not interfere with natural development of immunity (Haberkorn, 1986, Haberkorn and Stoffefuss, 1987, Grief 1999). In the present study Baycox (tohrazuril) was used in clinical hepatic coccidiosis in rabbits.

MATERIALS AND METHODS

Animals

Young (1-2 months old) New Zealand white rabbits (*Oryctolagus cuniculus*) of either sex weighing 2.0 to 3.0 kg were kept in the Department of Veterinary Parasitology, Punjab Agricultural University, Ludhiana for experimental studies. All the rabbits were found infected with *E. stiedae* as detected by faecal examination and of few dead rabbits by postmortem examination.

Clinical examination

The symptoms of the naturally infected animals were recorded daily. Individual faecal samples of rabbits were collected from rectum in small (2" x 2") polythene bags. The samples were then processed in the laboratory and examined by direct smear as well as saturated sugar floatation technique for the presence and identification of eimerian oocysts (Soulsby, 1982). Counts of coccidial oocysts per gram (OPG) of faeces were done using the McMaster technique to assess the intensity of infection prior and after treatment. Sporulation of oocysts was done in 2.5 % potassium dichromate solution at 25 °C (Hagen, 1958) as depicted in Fig. 1. The size of the oocyst was measured by using calibrated ocular micrometer. Histopathologically, livers of the dead rabbits were thoroughly examined for the gross lesions and liver tissues were collected in 10 % formalin. Tissues were processed as per standard technique and 5-6 µm thick paraffin embedded sections were cut for histopathological examinations after staining with haematoxylin and eosin (H. & E.).

Experimental design

The infected rabbits were divided into two groups. In group I (n = 18) rabbits were treated with 2.5 % tohrazuril (Baycox ,Bayer India Ltd., Bombay) at 25 ppm in drinking water for two days. The rabbits in group II (n = 6) were kept as infected untreated control. Assessment of therapeutic efficacy The therapeutic efficacy was assessed on the basis of absence of oocysts in the faecal samples, no clinical signs and mortality rate in treated rabbits as compared to infected untreated rabbits. The treated rabbits were clinically examined daily for any

improvement and the faecal samples were collected on days 0, 7, 14, 21 and 28 post-treatment and examined for the presence of oocysts per gram of faeces.

RESULTS

The infected rabbits showed the signs of anorexia, depression, brown watery diarrhoea, emaciation, rough hair coat, pendulous and distended abdomen with progressive weakness and death within 3-4 days. Jaundice was also reported in one case. The size of the sporulated oocysts varied from 32.75-40.7 x 17.10-21.7 μm (Fig. 1). At necropsy the liver showed hepatomegaly with numerous and scattered yellowish white nodules from 0.2 to 0.5 cm in diameter on the liver surface with distended gall bladder as gross changes (Fig. 2). A thick creamy white fluid exuded on cutting the nodules, microscopic examination of which revealed the presence of numerous oocysts and gametogonous stages. In most of the cases of hepatic coccidiosis fibrous tissue replacing 70-80 per cent of liver parenchyma was noticed. The distended gall bladders were full of oocysts. Dark green mucus was present in the intestine.

Histopathologically the lesions mainly confined to the liver and dilated bile ducts. The changes included hyperplasia and hypertrophy of the bile duct epithelium with different developmental stages of *E. stiedae*. The bile ducts were enlarged and had mainly two types of changes i.e. either they were having extensive proliferation of bile duct epithelium leading to formation of papilliform projections of epithelium into the lumen containing very large number of oocysts (Fig. 3) or they were highly dilated with flattened epithelium having no or minimum projections and the lumen full of numerous oocysts (Fig. 4). Most of the hepatic parenchyma around the peripheral areas was replaced with fibrous tissue and the bile ducts, and the remaining hepatic cells of the peripheral area showed degenerative inflammatory changes (Fig. 5). At places, dilation of central veins, sinusoidal dilation, atrophy of cords (Fig. 6) and telangiectasis were noticed. Some of the bile ducts were quite small and contained different development stages of *E. stiedae* (Fig. 7) surrounded by chronic inflammatory reaction, fibrosis and even giant cells (Fig. 5). Proliferation of biliary epithelium along with numerous oocysts with in the lumen of bile ducts causes stasis of bile (Fig. 8).

Baycox (tofrazuril) at 25 ppm in drinking water for two days was highly effective against hepatic coccidiosis as observed on OPG, clinical signs and prevention of mortality in clinical cases of hepatic coccidiosis. The drug not only effectively suppressed the clinical signs of

Clinical hepatic coccidiosis

coccidiosis but also prevented shedding of oocysts in the faeces of infected and treated rabbits in group I (Table 1). The rabbits of group II infected unmedicated showed high oocyst output as well as 100 per cent mortality by day 7. No adverse reactions were observed with applied dose regimen of tofrazuril.

Table 1. Oocysts per gram (OPG) of faeces (1×10^3) in *Eimeria stiedae* infected and tofrazuril-treated rabbits.

Groups	No. of animals	Parameter	Days post treatment											
			0	1	2	3	4	5	6	7	14	21	28	
1*	18	OPG	8.40	11.30	7.35	6.04	2.13	1.40						
		(mean \pm SEM)	\pm 2.59	\pm 2.58	\pm 3.47	\pm 4.43	\pm 2.65	\pm 2.29	- (18)	- (18)	- (18)	- (18)	- (18)	
					(4)	(9)	(13)							
2**	6	OPG	9.35	12.24	9.48	11.27	10.90	11.80	11.25					
		(mean \pm SEM)	\pm 1.44	\pm 2.39	\pm 1.67	\pm 2.31	\pm 2.37	\pm 2.69	\pm 1.39	-	-	-	-	
		No. of rabbit died	0	1	0	1	0	1	1	2	0	0	0	

* Rabbits infected and treated.

** Rabbits infected unmedicated

Figures in parentheses indicate number of rabbits negative for OPG.

DISCUSSION

The size and weight of livers of dead animals increased due to intense proliferation of biliary epithelia resulting into pressure atrophy of the hepatic parenchyma. This hepatomegaly which is characteristic of this disease (Barriga and Arnoni, 1979) results in enlarged and pendulous abdomen. The proliferation of the bile duct epithelium as seen in many of the bile ducts (Fig. 3) might be due to the predilection and proliferation of the *E. stiedae* organisms within the epithelium, whereas extensive dilation with little or no proliferation of the bile duct epithelium (Fig. 4) might indicate the cell turn over which was also proved by the fact that these ducts contained more number of oocysts as compared to the ones that had conspicuous proliferation. The wide spread sinusoidal dilation, associated with fibrosis in and around the cords as well as telangiectasis might be attributed to the obstructed hepatic blood flow especially

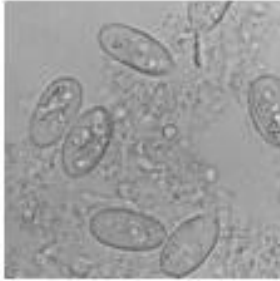


Fig. 1

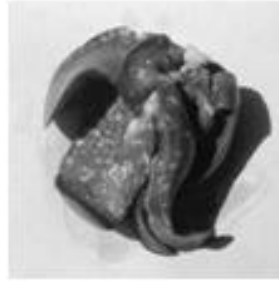


Fig. 2

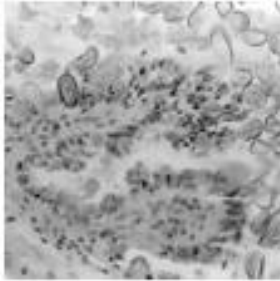


Fig. 3

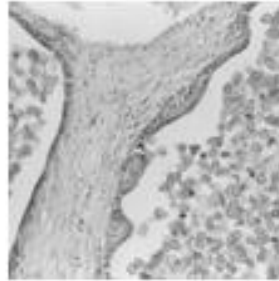


Fig. 4

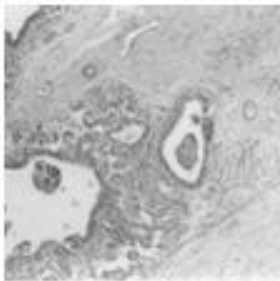


Fig. 5

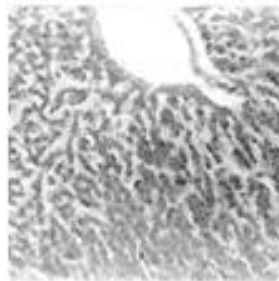


Fig. 6

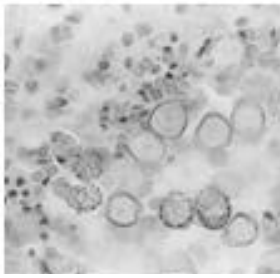


Fig. 7

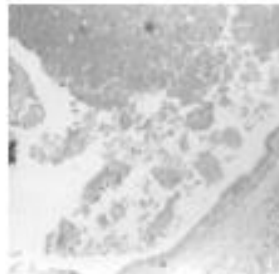


Fig. 8

Fig. 1. Flotation preparation of rabbit faeces showing sporulated oocysts of *Eimeria stiedae* x 300.

Fig.2. Liver-a surface view showing yellowish white nodules distributed throughout the swollen liver..

Fig.3. Papillomatous hyperplasia of bile duct epithelium and a number of oocysts. H&E x 300.

Fig.4. Section of liver showing distended bile ducts with minimal proliferation containing numerous oocysts. H & E x 150.

Fig.5. Inflammatory changes in the peri-ductular connective tissue of liver. H&E x 75.

Fig.6 Section of liver showing dilatation of central vein, sinusoids and necrosis of hepatocytes. H&E x 150.

in the portal veins by immensely proliferating and dilating bile ducts.

The stagnation of the blood flow would also result in decreased hepatocellular degeneration and atrophy of the cords. Rosmini and Simoni (1979) reported hyperplasia of infected biliary epithelium with degeneration of all cytoplasmic structures. If the continuity of epithelium of bile ductules is broken, the coccidian organisms would act as foreign bodies and it might involve typical foreign body granuloma. Mehamoud and Ibrahim (1989) reported granulomatous hepatitis in coccidial infection in which coccidial oocysts were observed in the central region of granuloma which later resulted in destruction and fibrosis of large areas of hepatic lobules. The stasis of bile due to biliary obstruction by proliferation of bile duct epithelium along with numerous oocysts within the bile duct lumen results in obstructive jaundice (Sanyal and Sharma, 1990). The hepatic coccidiosis caused severe damage to the liver, thus recovered rabbits have usually shown stunted growth (Morton-Smith, 1997).

The dose of 25 ppm of Baycox for two consecutive days was found to be highly effective which is in agreement with the previous report of Zurliiski and Vladiminova (1988) against intestinal coccidiosis of rabbits. This dose resulted into zero OPG while James and Campbell (1991) reported that two 48 hour treatments with 25 ppm of tohrazuril in water, 5 days apart reduced oocyst output to zero approximately 40 days in hepatic and intestinal mixed infection. Zero OPG on days, 7, 14, 21 and 28 post treatment (Table 1) with Baycox indicated an indirect effect on the free living stages due to severe suppression of or the killing effect on the proceeding stages. Thus it is concluded that Baycox given at 25 ppm for two days was an effective method of reducing oocysts due to hepatic coccidial infection to nil. Further investigations would be needed to assess the effectiveness of tohrazuril in sub-clinical coccidiosis of rabbits as well as appropriate timing for employing the treatment.

ACKNOWLEDGEMENTS

Authors are thankful to Professor-cum-Heads, Departments of Veterinary Parasitology and Veterinary Pathology, Punjab Agricultural University, Ludhiana for faculties provided

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