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## Eimeriidosis and Pathological Findings in New Zealand White Rabbits

Mohammad Yakhchali and Aliasghar Tehrani

Department of Pathobiology, Pathology Division, Faculty of Veterinary Medicine,  
Urmia University, Urmia, Iran

**Abstract:** An investigation into eimeriidosis of New Zealand White rabbits was carried out in existence rabbitries of northwest region, Urmia suburb, Iran. Four hundred and thirty six rabbits in two rabbitries which were from less than six months to over 12 months old were subjected to examination. Fecal samples were collected and performed to flotation technique using for demonstrating the presence of oocysts and sporulation of oocysts. The values of the Oocysts Per Gram (OPG) of feces vary from 5000 to 60000. Of these, 60% had 5000-29000 OPG. Of infected animals, 53 (55.8%) and 42 (44.2%) were females and males, respectively. Mixed infections were common and 67% of the animals carrying 2-4 different species. Six species of *Eimeria* were identified. The infection of livers with *Eimeria stiedae* was 19.9%. There was high rate of infection of intestinal contents (80.1%), especially, with *E. magna* (34.8%), *E. irresidua* (20.8%), *E. perforans* (9.8%), *E. media* (8.3%) and *E. piriformis* (6.4%). Histopathology changes were indicative of inflammatory reactions brought about by parasitic infection with *Eimeria* species and its consequent irritating effects on the liver and intestine.

**Key words:** *Eimeria* spp., intensity, pathology, rabbit

### INTRODUCTION

The coccidia comprise of a large group of obligatory intracellular parasites (Duszynski *et al.*, 1999). The different species of *Eimeria* has been reported by Eckert *et al.* (1995). Eleven species of intestinal, caecal or colonic coccidia were documented (Flecknell, 2000) and have varying degrees of pathogenicity. Their role in intestinal pathology have been recently amplified by the epizootic rabbit enteropathy syndrome (Coudret *et al.*, 2000).

The most important species of rabbit coccidia is *E. stiedae* (Al-Rukibat *et al.*, 2001). This species invades and develops in hepatobiliary epithelial cells of domestic rabbits. It causes intensive cholestasis and biliary cirrhosis (Hanada *et al.*, 2003). Coccidiosis is a common, widespread problem in commercial operations and research. It is an important economic and disease of the young rabbit, especially, in breeding and rearing establishments where sanitation measures are poor. However, an outbreak of disease is not uncommon under natural conditions where the type of habitat is found.

The first study on rabbit coccidiosis has been done in central part of Iran from four rabbitries and eight species of *Eimeria* were identified (Niak, 1981). This study was undertaken to determine diversity of *Eimeria* species in New Zealand White (NZW) rabbit. Necropsies were

performed to measure histopathological changes due to eimeriidosis, throughout whole intestine length and liver on the basis rabbit rearing in Iran.

### MATERIALS AND METHODS

Rabbit populations (436) were sampled from two rabbitries in Urmia suburb, Iran, in 2005. They were housed intensively on litter system. All of them did not receive any anti coccidian treatment such as coccidiostats. They were 145 (33.3%) rabbits with less than six months old, 215 (49.3%) rabbit with 6-12 months old and 76 (17.4%) rabbit over 12 months old. During investigation, rabbits were fed by concentrates and vegetables *ad libitum*.

**Parasitological procedure:** Samples were chosen randomly and put into a plastic container, separately; with a lid. The data pertaining to the sex, age and feces consistency was recorded. A part of each sample (3 g) was mixed with tap water (42 mL) (Hendrix, 1998). Mixture was subjected to centrifugal sedimentation (1500 rpm for 3 min) and flotation technique using standard sheather solution (specific gravity 1.12) for detection of *Eimeria* oocysts, which then counted by the modified McMaster technique (Anon, 1984). The intensity of infection was estimated in terms of Oocysts Per Gram (OPG coefficient)

of feces. Fecal culture was the method which described by Duszynski and Wilber (1997). Contents were collected and processed individually (duodenum, jejunum, ileum, caecum, colon) using dichromate potassium (2.5%  $K_2Cr_2O_7$ , w/v) to allow oocysts to sporulate. Most sporulated oocysts were identified and traced to species using cover slip flotation with modified Sheather's sucrose solution (Hendrix, 1998). Phenotypic characters used for identifying eimeriid coccidia including morphology and morphometry of the oocysts (Eckert *et al.*, 1995).

**Histopathological procedure:** Twenty four (25.3%) young infected rabbits with clinical symptoms such as grow poorly, diarrhea and heavily oocyst shedding was sedated by Ketamine and euthanized using Sodium pentobarbital (100 mg  $kg^{-1}$ , IV) a method consistent with recommendation of panel on Euthanasia of the American Veterinary Medical Association. Wet mounts of liver foci and gall bladder were provided and observations recorded. Then, intestine and liver were subjected for histological examination. The histological sections were collected from liver and intestine, fixed in 10% buffered formalin and processed routinely, sectioned at 4-5  $\mu m$ , stained with hematoxyline and eosin (Carlton and McGavin, 1995). Slides were examined under light microscope, observations were recorded and compared.

## RESULTS AND DISCUSSION

**Clinical findings:** The results of this study are the first to report *Eimeria* infection in NZW in this part of Iran. The values of the oocysts per gram of feces (OPG) vary from 5000 to 60000. Of these, 60% had 5000-29000 OPG. Mixed infections were common and 67% of the animals carrying 2-4 different species. Of infected animals, 53 (55.8%) and 42 (44.2%) were females and males, respectively.

Six species of *Eimeria* were identified. There was high infection of intestinal contents with pathogenic species, i.e., *E. magna* (34.8%), *E. irresidua* (20.8%), *E. media* (8.3%) and *E. piriformis* (6.4%) and low pathogenic species, i.e., *E. perforans* (9.8%). These results are similar to the reports of former Balicka-Laurans *et al.* (1990), Darwish and Golemansky (1991), Gurpata and Khahra (1997). In another investigation, Gres *et al.* (2003) reported the same finding in wild rabbit (*Oryctolagus cuniculus*). Five *Eimeria* species were reported by Toula and Ramadan (1998) and 90% of the examined rabbits were positive and mixed infection with 2-3 *Eimeria* species was most frequent. According to the Catchpole and Norton (1979) and Peeters *et al.* (1985) the *E. media*, *E. magna* and

*E. perforans* were occurring more frequently, whereas *E. irresidua* and *E. piriformis* are less common.

In weanling rabbits, the usual presentation was diarrhea. In older ones, more frequently intestinal stasis and illus occurred and little feces were passed. The development of *E. piriformis* was in the large intestine, including the colon and caecum. *E. magna* and *E. media* infected the jejunum and the ileum. *E. irresidua* and *E. media* have been multiplied in whole small intestine. The *E. perforans* was infected the duodenum and the jejunum. The *E. magna* and *E. irresidua* were seen most frequently and *E. piriformis* was comparatively less common.

In present study, infected livers with *Eimeria stiedae* (Pellérdy, 1974) were 19.9%. In Brazil, 48% of rabbits that died in breeding farms and 64% of rabbits slaughtered in the abattoirs were infected with *E. stiedae*. In contrast, Zarzara *et al.* (1989) found that the coccidial infection of livers was nearly 0.1%. Incidence of coccidiosis varies and has been decreased in laboratory rabbits. It depends on geographical location (Soulsby, 1986; Barriga, 1979). In earlier studies by Catchpole and Norton (1979) and Peeters *et al.* (1981) they found that *E. stiedae* was not common in rabbits. Whereas our results were indicating that the most important species of rabbit coccidiosis was *E. stiedae* (18.9%).

**Pathological findings:** At necropsy, the small intestines were distended and filled with gray-green semisolid ingesta. Undigested food pellets were present in the colon. The small intestine was congested and edematous with foci of hemorrhages scattered on the mucosa. The ileum was bruised and pale and segmentation was appeared very clearly, especially, close to the caecum. The most constant feature was the emptiness of the caecum.

From the histological point of view, there was atrophy of the enterocytes lining the villi of the mucosa due to *Eimeria* species, which were shortened. Furthermore, the number of parasitized epithelial cells in comparison to the whole population of epithelial cells was low. Intestinal coccidiosis interferes with intestinal function. It causes villous atrophy (Peeters *et al.*, 1985) and impairs intestinal motility (Fioramonti *et al.*, 1981). The Lieberkuhn's glands were collapsed and decreased in number and varied in size. Other findings of histopathology of intestine coccidiosis thus lend support for the observations of Wang and Tsai (1991) and Carlton and McGavin (1995). Diffuse leukocytic infiltration, which was mainly comprised of lymphocytes, plasmacells and eosinophils, were observed. Macrogamont and microgamont were seen (Fig. 1a and b).

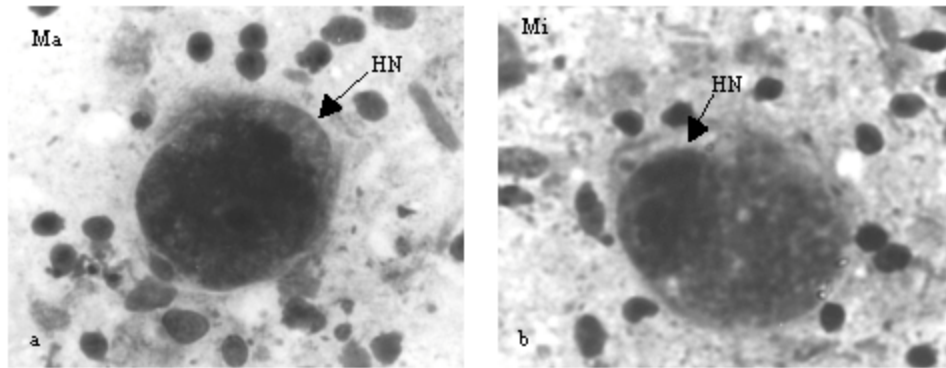


Fig. 1: Intestine of a natural infected rabbit with *Eimeria* species, Macrogamont (1250x) (a) and Microgamont (1250x) (b); HN, host nucleus; Ma, macrogamont; Mi, microgamont

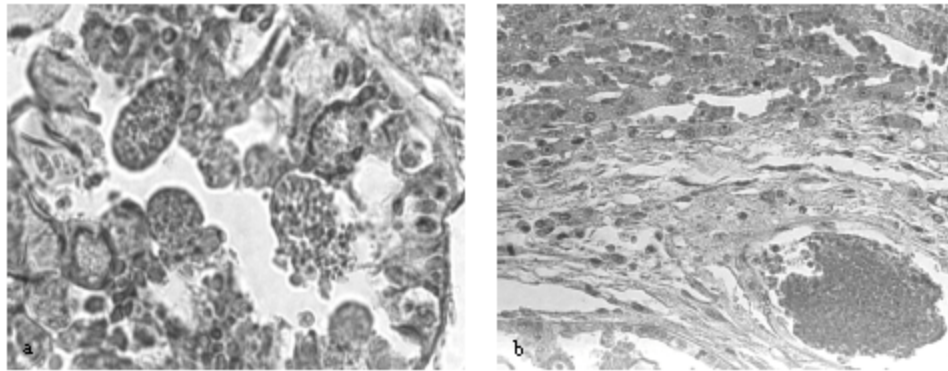


Fig. 2: Liver and bile duct of a natural infected rabbit with *Eimeria stiedae*, formed schizonts within the epithelium of bile duct (a), hyperemia and intercellular hemorrhage (b); H and E; 500x

Macroscopically, the liver was enlarged and had multiple 2 to 4 mm diameters. The parenchyma was congested and edematous. Wet mounts of liver foci and gall bladder showed all forms of the parasite.

Histological findings in the liver consisted epithelium hyperplasia of bile ducts, enlargement of bile ducts with infiltration of inflammatory cells (eosinophils, lymphocytes and plasmacells) and moderate number of erythrocytes. Irregular yellowish foci of necrosis of parasitized bile ducts were found. Merozoites and sporozoites were not found, more likely, due to the late stage of infection in rabbit. The Lumina of the bile ducts were filled with oocysts (Fig 2a and b). No pathological differences were seen among infected rabbits. The results have completed the pathological findings of the previous study carried out by Barriga(1981), Chen *et al.* (1972) and Gomez-Bautista *et al.* (1987).

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