Pathological Study on an Outbreak of Hepatic Coccidiosis in Rabbit Farm

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Abstract

Hepatic coccidiosis was recorded in a commercial rabbit farm. Clinically anorexia, rough hair coat, distended abdomen, watery to semisolid diarrhoea, jaundice and sudden death was recorded in rabbit kits with 58.14 % morbidity and 62 % mortality rate. Grossly, the abdominal cavity contained clear straw coloured fluid. Liver showed diffuse severe congestion to pale, enlargement and yellowish grey nodules. Liver impression smear revealed high cellularity and high numbers of coccidial organisms intermixed with hepatobiliary parenchymal cells. On parasitological examination of liver sample, bile sample and faecal sample, oocyst and sporulated oocysts of Eimeria stiedae was identified. On histopathological examination, liver showed severe papillary hyperplasia of the lining epithelium of bile ducts contained numerous coccidial oocysts and developmental stages of the coccidium. In many areas, the basement membranes were thickened and areas of detachment of papillary projection of ductal epithelium from the wall Multiple areas of coagulative necrosis of hepatocytes with infiltration of lymphocytes were also observed. The other histopathological changes such as subacute tracheitis, subacute pneumonia. Focal subacute myocarditis, subacute catarrhal gastritis, subacute catarrhal enteritis and mild lymphoid depletion in spleen and lymph nodes were also recorded. Based on pathological and parasitological examination, the present outbreak was diagnosed as hepatic coccidiosis in rabbits.

Keywords: *Argulosis, Pathomorphology, Fresh water fish*

Introduction

Hepatic coccidiosis is caused by *Eimeria stiedae* and it is one of the most pathogenic coccidian protozoans in domestic rabbits causing high mortality (Darwish and Golemansky, 1991; Becha and Devi, 2014). It is reported in different parts of India (D'Souza *et al*., 1992; Jithendran and Bhat, 1995; Magrav *et al*., 2010; Palanivel *et al*., 2013 Becha and Devi, 2014). There is a strong relationship between the infection of coccidiosis and the host age; they believe that the infection decreases with increasing age of the host. Young rabbits are more susceptible (Naimi *et al*., 2012). Massive infection shows meteorism, in appetence, depression, brown watery diarrhea or constipation, emaciation, rough hair coat, pendulous and distended abdomen and hepatomegaly, progressive weakness, Jaundice and death. (Bhatia, 2000; Naimi *et al*., 2012). The histopathological changes showed biliary hyperplasia with different developmental stages of *Eimeria stiedae* in the epithelial cells, cholangitis and peribiliary fibrosis with newly formed bile ductules (Naimi *et al*., 2012).

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The aim of the present study was to investigate outbreak of hepatic coccidiosis in a rabbits farm.

Materials and Methods

A farmer brought five rabbit kits for the postmortem examination with history of anorexia, rough hair coat, watery to semisolid diarrhoea, jaundice and sudden death. The farm with capacity of seventy six adult domestic rabbits and 182 rabbit kits of various age group (15 days to 75 old). All the adult rabbits and young ones were fed with different dry cereals (e.g. broken corn, millets, rice, etc.) and green fodder, cabbage leaves. 150 rabbit kits showed clinical signs for the last 10 days and 93 rabbit kits of 45 to 75 day old age died within 5 days. Fecal samples collected from the rabbits (females and males) were examined by centrifugal flotation with saturated sugar solution to detect *E. stiedae* oocysts (Heelan and Ingersoll (2002). Collected oocysts were mixed with 2.5% potassium dichromate for sporulation of oocysts. Each liver and gall bladder was removed and examined for nodules typical (characteristic) of infection with *E. stiedae*. Liver impression smears and sediments of bile were stained with Leishman stain. Faecal samples from the collected rabbits were submitted to Division of Veterinary Parasitology for parasitological examination. After the complete necropsy, representative tissue samples from the liver, gall bladder and various other organs were collected and fixed in 10% formalin for the histopathological studies. After proper fixation, tissue blocks were embedded in paraffin. Thin (5 micron) sections were routinely prepared and stained with haematoxylin and eosin.

Results

The clinical signs recorded were anorexia, watery to semisolid diarrhoea, progressive emaciation, jaundice and death**.** The morbidity and mortality rate was 58.14 and 62 % respectively. Grossly, the abdominal cavity contained clear straw coloured fluid of about 5-10ml. Liver showed diffuse severe congestion to pale, enlargement, yellowish grey, round, discrete and irregular shaped nodules of 1-5 mm in diameter. On cut section of the liver, yellowish white cheesy contents within the nodules. Thoracic cavity contained about 3-4 ml of straw coloured transparent fluid. Gall bladder contained greenish yellow, thick bile. Lungs revealed diffuse moderate congestion and patchy areas of reddish brown firm areas. The cut section of those areas sank into water. Kidneys showed diffuse moderate congestion and enlargement. Stomach was empty and its mucosa revealed diffuse mild congestion. Intestine revealed patchy areas of mild serosal and mucosal congestion.

Liver impression smear revealed high numbers of coccidial organisms intermixed with hepatobiliary parenchymal cells and low numbers of inflammatory cells. The coccidial organisms were in various stages of development that included early gametogonous stages, microgametocytes, macrogametocytes, and fully formed oocysts. Oocysts were predominantly ovoid to ellipsoidal and various sizes. The bile sediment smears revealed many oocysts of narrowly oval in

shape with cap and smooth wall, each contains an undeveloped zygote surrounded by yolk cells. Based on parasitological examination of liver sample, bile sample and faecal sample, the oocyst and sporulated oocysts were morphologically identified as *Eimeria stiedae*.

On histopathological examination, liver showed diffuse severe congestion of sinusoids, central veins, multifocal haemorrhages and diffuse mild vacuolar degeneration of hepatocytes. The bile ducts were greatly thickened and distended. Severe papillary hyperplasia of the lining epithelium of bile ducts forming finger-like projections in to the lumen of the bile duct. Biliary ducts were filled with sloughed biliary epithelial cells with numerous coccidial oocysts and developmental stages of the coccidium. In many areas, the basement membranes were thickened and in some areas detachment of papillary projection of bile ductal epithelium from the wall along with the overlying coccidian contained epithelial cells in the lumens of ducts. Bile duct hyperplasia, chronic periportal hepatitis characterized by periportal and periductular fibrous tissue proliferation with mononuclear cell infiltration were also recorded. Multiple areas of coagulative necrosis of hepatocytes with infiltration of lymphocytes were also observed. (Fig.1-6).

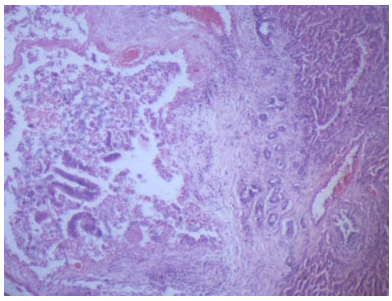

Fig. 1: Liver showed bile duct hyperplasia with periductular fibrosis H&E X20x

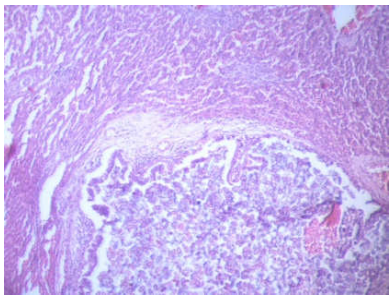

Fig. 2: Bile duct showed papillary hyperplasia with intra luminal coccidial oocyst H&E X100x

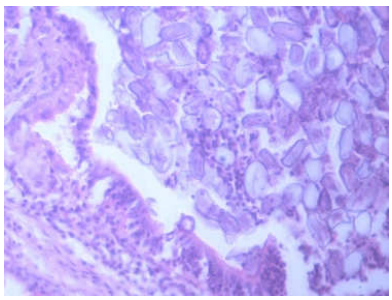

Fig. 3: Lumen of bile duct revealed many oocyst H&E X400x

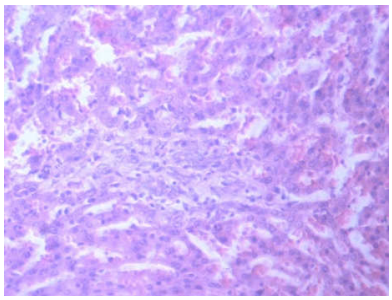

Fig. 4: Liver showed blieduct degeneration, necrosis, MNC infiltration and oocysts H&E X400x

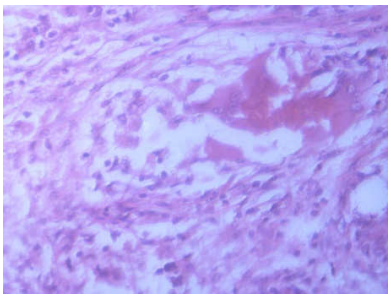

Fig. 5: Liver showed necrosis of hepatocytes H&E X400x

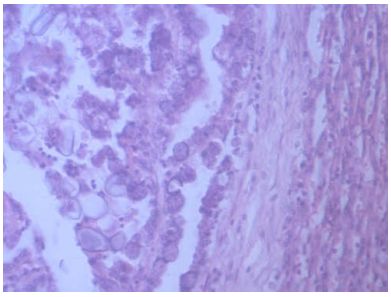

Fig. 6: Liver showed thickening of basement membrane and various stages of Eimeria stiedae in bile ductular epithelial cellsnecrosis of hepatocytes H&E X400x

Trachea showed subacute tracheitis. Lungs revealed multifocal subacute pneumonia. Heart revealed diffuse mild congestion, multifocal mild haemorrhages, focal subacute myocarditis. Spleen and lymph nodes showed diffuse mild lymphoid depletion. Stomach showed subacute catarrhal gastritis. Intestine revealed subacute catarrhal enteritis. Based on pathological and parasitological examination, the present outbreak was diagnosed as hepatic coccidiosis in rabbits.

Discussion

Rabbit is the only animal that suffers from hepatic form of coccidiosis leading to severe economic losses (Darzi *et al*., 2007). Coccidal infection is affected by the host age; the highest incidence was in 2-months rabbits and then the infection rate decreased as the age increased. The high level of susceptibility of infection in young rabbits may be due to their immune, feeding and reproductive status (Hobbs *et al*., 1999; Hagen, 1958; Erdogmus and Eroksuz, 2006). Hepatic coccidiosis which caused by *E. steidae* is a primary disease of young rabbits. In rabbits, hepatic coccidiosis occurs due to ingestion of sporulated oocysts which is having four ovoid sporocysts containing 2 sporozoites each. The sporozoites penetrate small intestinal mucosa and pass via the mesenteric lymph nodes and hepatic portal system to the liver where they enter the epithelial cells of the bile duct becoming trophozoites and then schizonts. (John *et al*., 1999). Oocysts pass out in the bile and appear in the faeces 18 days after infection, sporulation occurs in three days (Gardiner *et al.*, 1998). Clinical signs, morbidity and mortality rate were higher in the present farm was in accordance with the findings of *Al-Mathal* (2013) and Becha and Devi (2014). In the present outbreak, young rabbit kits of age group 45 to 70 days old were died. This hepatic coccidiosis caused severe damage to the liver and severely affect young rabbits and led tohypoprotaememia, jaundice, ascites and death (Wang and Tasi, 1991; Naimi *et al*., 2012). Bautista *et al*., 1987 reported that clinical effects of hepatic coccidiosis are more severe in young ones and resistance develops in age advances. Adult remain carriers and are potential threat to susceptible young ones (Wang and Tasi, 1991). The highest incidence was in 2-

months rabbits and then the infection rate decreased as the age increased. The high level of susceptibility of infection in young rabbits may be due to their immune, feeding and reproductive status. This observation is consistent with the results previously reported (Naimi *et al*., 2012). On liver impression smear, the coccidial organisms were in various stages of development that included early gametogonous stages, microgametocytes, macrogametocytes, and fully formed oocysts. Similar findings were recorded by Al-Rukibat *et al*. (2001).

Histopathologically, the wide spread sinus dilation, haemorrhages associated with fibrosis in and around the bile duct might be attributed to the obstructed hepatic blood flow especially in the portal veins by immensely proliferating and dilating bile ducts. The stagnation of the blood flow results in hepatocyte cellular degeneration and atrophy of the cords. These histopathological observations are in agreement with those described by others (Singla et al., 2000; Naimi *et al*., 2012). If the hepatic continuity of epithelium of bile ductules is broken the coccidian organism or oocyst would act as foreign bodies it might involve typical foreign body granuloma. Granulomatous hepatitis in coccidial infection in which coccidial oocysts were observed in central region of granuloma which later results in destruction and fibrosis of large area of hepatic lobules. The hepatic parenchyma showed areas of fibrosis with obstructive jaundice and that occurred due to massive necrosis of parenchyma leading to post-necrotic scarring. The bile pigment deposition was due to obstruction of the main ductal system from local swelling and jaundice is almost always present and that in agreed with previous studies (darzi *et al*., 2013). In the present study, increase in the thickness of the basement membrane indicating its involvement in the pathogenesis of the papillary hyperplasia. Chen *et al.*, (1972) recorded thickened basement membrane appears to be a defensive act on part of the host against coccidial infection which impede the transfer of essential nutrients to the overlying epithelium and induce regression and degeneration of a hyperplastic epithelium. Basement membrane in the form of loop-like structures and subsequent detachment of this loop along with the overlying hyperplastic epithelium showed that basement membrane has a role in defence mechanis (Darzi *et al*., 2007). Detached basement membrane structures were seen in the lumens of the ducts thereby shedding the epithelium containing developmental stages of the coccidian. Besides, the existence of a basement membrane seems to facilitate the orientation of the newly formed epithelial cells in the epithelialisation process (Giacometti and Parakkal, 1969; Darzi *et al.*, 2007)

Histopathological observations in this study are in agreement with those described by others (Khalifa *et al*., 1998; Toula, 2000; Omar, 2004; Darzi *et al*., 2007). Coccidial infection is affected by the host age; This hepatic coccidiosis caused severe damage to the liver and it is more pathogenic in young rabbits and led

to death among these animals. The proliferation of the bile duct epithelium might be due to the predilection and proliferation of the *E. stiedae* within the epithelium, These histopathological lesions confirmed the results of parasitological examination of faeces which revealed the presence of large number of oocysts (Omar, 2004). In the present study pathological lesions such as subacute tracheitis, subacute pneumonia, subacute myocarditis, subacute catarrhal gastritis, subacute catarrhal enteritis and lymphoid depletion in spleen and lymph nodes could be possibly due to *Eimeria stiedai* and it could possibly cause immunosuppression in lymphoid organs. Darzi *et al*., (2007) recorded congestion in lungs and cellular swelling and vacuolar degeneration of the parenchymal cells of kidneys. In conclusion, based on pathological and parasitological studies, present outbreak was recorded as hepatic coccidiosis.

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