Study on impression smears of hepatic coccidiosis in rabbits

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Received: 15 September 2014 / Accepted: 13 October 2014 / Published online: 30 October 2014
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Abstract Hepatic coccidiosis is a contagious and lethal disease condition in rabbits. The disease was recorded in six rabbits suffering with watery diarrhoea. Clinically, affected rabbits showed decreased growth rate, anorexia, debilitation, diarrhea and rough hair coat. Examination of the faecal samples revealed the presence of unsporulated oocysts of *Eimeria* spp. After sporulation *Eimeria stiedae* oocysts were identified. Postmortem examination revealed hepatomegaly with presence of discrete yellowish-white nodules on the surface of the liver. Impression smears from the liver revealed the presence of numerous developmental stages of *E. stiedae* corresponding with the stage of the liver lesion and also represent the histological changes of the liver. Rabbits were treated with a combination of sulphaquinoxaline and diaveridine for five days.

Keywords *Eimeria stiedae* · Impression smears · Hepatic coccidiosis · Rabbits · Y.S.R. District

Introduction

In the present days, rabbits are used for good quality protein diet, pets in the houses and used for the research

studies as laboratory animals (Sivajothi et al. 2013a). Coccidiosis is a ubiquitous protozoan infection of animals seriously impairing their growth and it causes significant mortality in domestic rabbits (El-Akabawy et al. 2004). Two forms of coccidiosis were recognized in rabbits; hepatic coccidiosis and intestinal coccidiosis. The hepatic coccidiosis is due to *Eimeria stiedae* that cause severe damage to the liver and leads to death (Oncel et al. 2011). Young rabbits are most susceptible; however, infected adults become carriers of the disease and are a source of infection. Most of the coccidian infections are believed to be mild and although mild infections are often clinically inapparent, severe infections may be extremely pathogenic and may result in death.

Diagnosis of hepatic coccidiosis is generally done by analysis of faeces of suspected animals. Routine diagnosis of hepatic coccidiosis in live animals can be done by examination of faecal sample and subjecting it to further sporulation studies for confirming the species (Sivajothi et al. 2013b). Impression smear examination is an old method for diagnosis of hepatic coccidiosis. But, literature regarding the study on the detailed impression smear staining was very little in Andhra Pradesh of India. The present communication was a study based on examination of fresh faecal samples and impression smears collected from the necropsy of rabbits affected with hepatic coccidiosis.

Materials and methods

Six rabbits with diarrhoea were presented to the Teaching Veterinary Clinical Complex, Proddatur from a rabbit farm in Y.S.R. District of Andhra Pradesh. Affected rabbits showed the symptoms of anorexia, debility, listlessness,

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distended abdomen, soiling of hind quarters with faeces and yellowish mucus membranes along with muscle wasting. Rabbits present in the farm were belongs to the New Zealand and Grey Giant breeds comprised of varying ages- ranging from less than a month old to over 2 years. Up on enquiry the owners of the rabbits revealed that the two rabbits were died showing the similar symptoms in the morning. Died and affected rabbits had age between 2 and 4 months age.

Fresh faecal samples from the affected rabbits were collected into sterile containers for processing. Collected faecal samples were examined by centrifugal flotation with saturated salt solution to detect *E. stiedae* oocysts according to the standard method (Al-Mathal 2008). Collected oocysts from the positive samples were mixed with 2.5 % potassium dichromate for sporulation of oocysts according to the standard method (Toula 2000). By conducting the necropsy, liver and intestines were collected for further examination. Impression smears were made from the cut surface of the liver, crushed nodules of the liver and sediments of bile. These were stained with Leishman's staining.

Results

In the necropsy examination, the liver was found to be enlarged and yellowish white nodules of 1 to 5 mm diameter was distributed throughout the surface. The bile ducts were dilated and gall bladder was distended.

Unsporulated oocysts were identified in the faecal samples of affected rabbits (Fig. 1). Unsporulated oocysts of *E. stiedae* was identified in the impression smears from liver lesions as well from the bile. The oocysts have a smooth yellowish orange coloured wall, ovoid in shape measuring 32–40 μm length, 16–21 μm width with a micropyle. In the present study, sporulation time of 72 hours was observed at room temperature. Sporulated oocyst is the infective stage of coccidian parasites. It's oval in shape with cap and double wall, each contains four sporocysts (Fig. 2). The Sporulated oocyst measures 30 μm in length and 20 μm in width. The impression smears from the liver cut surface included 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 predominated and were ovoid to ellipsoidal shape (Fig. 3).

Macrogametes appeared in rounded to oval shape, ranging from 20 to 23 μm in width and 25 to 34 μm in length and filled with homogeneous pink to bluish

cytoplasmic granules with a row at the periphery, referred to as the wall forming bodies (Fig. 4). The microgametocytes were distinguishably large and rounded in shape, measuring up to 45 μm in diameter and filled with a large number of purple rounds to comma shaped microgametes (Fig. 5). Hepatocytes revealed vacuolar degeneration (Fig. 6). Very low numbers of inflammatory cells observed were small reactive lymphocytes, plasma cells and heterophils. A low to moderate number of erythrocytes was also seen. The cytologic findings were suggested that the rabbits were suffering with *E. stiedae* infection (hepatic coccidiosis) and associated bile ductular hyperplasia, with minimal hepatocellular vacuolar degeneration and intracellular cholestasis.

Based on the postmortem and impression smears examination, condition was diagnosed as hepatic coccidiosis. All the rabbits were treated with a combination of sulphaquinoxaline and diaveridine (Rancox powder) at the rate of 1 g/litre of drinking water for 5 days and advised to repeat the treatment after 5 days.

Discussion

Hepatic coccidiosis in domestic rabbits was a world wise disease condition. The present study record the most important coccidian parasite of domestic rabbits, *E. stiedae* in the Y.S.R. District of Andhra Pradesh in India from the impression smears of the liver.

The life history of coccidia such as *Eimeria* includes both asexual (schizogonous) and sexual (gametogonous) reproduction (Gardiner et al. 1998). The released sporozoites penetrate the epithelial cells lining the intestinal wall and initiate scizogony (asexual multiple division). When the merozoites are mature, the host cell ruptures and releases the merozoites, which in turn seek a new cell to infect or repeat merogony again. The number of merogony

Fig. 1 Unsporulated oocysts ($\times 400$ with $\times 4$ camera magnification)

Fig. 2 Sporulated oocysts ($\times 400$ with $\times 4$ camera magnification)

Fig. 3 Oocysts in liver impression ($\times 400$ with $\times 4$ camera magnification)

Fig. 4 Macrogametocyte in impression smear ($\times 1,000$ with $\times 4$ camera magnification)

generations is species specific and can vary from two to four generations. The last generation of merozoites enter in to the host cells and develops as gametes during gamogony, the sexual reproductive stage. The macrogametocytes (female gamonts) enlarge, store nutrients, and cause

Fig. 5 Microgametocyte in impression smear ($\times 1,000$ with $\times 4$ camera magnification)

Fig. 6 Hepatocytes with vacuolar degeneration ($\times 100$ with $\times 4$ camera magnification)

hypertrophy of the host cell. When mature, they are called macrogametes and contain many hyaline granules (wall-forming bodies) at their periphery. The microgametocytes (male gamonts) undergo repeated nuclear divisions and become multinucleated; each becomes part of a biflagellated microgamete. The microgametes, freed by rupture of the host cell, seek out and penetrate macrogametes. Fertilization triggers formation of the cyst wall around the resulting zygote, producing the oocyst. When the wall is fully formed, the oocyst bursts from its host cell and is leaves the host via its faeces. Once outside the host, the oocyst must undergo sporogony in order to become fully mature (Gardiner et al. 1998). The oocyst divides twice to form four sporocysts and is ready to infect a new host.

The unsporulated oocysts are narrowly oval in shape with cap and smooth wall, each contains an undeveloped zygote surrounded by yolk cells. This stage present in the rabbit faces after the infection. The sporulated oocyst is ovoid to ellipsoidal and had flat micropylar end and a smooth bright-pink wall (Al-Rukibat et al. 2001). Hepatic coccidiosis can be diagnosed by identifying oocysts in the

feces, based on size, shape and structure. Oocysts must be differentiated from those of the intestinal coccidia, which may also be present in rabbits in large numbers without causing serious clinical signs.

This isolate of *E. stiedae* multiplies in the epithelial cells of bile ducts and is considered as a pathogenic organism (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. The hepatic coccidiosis caused severe damage to the liver, particularly in young rabbits and lead to death (Yakhchali and Tehrani 2007). Al-Mathal (2008) noted that the hyperplasia and enlargement of bile ducts and infiltration of inflammatory cells (eosinophiles, lymphocytes and plasma cells) were evident in rabbit hepatic coccidiosis. 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 and Eroksuz 2006). Hyperplasia of bile duct epithelium is accelerated along sexual phase and epithelial damage and desquamation will prevail during the invasion of merozoites and schizonts (Chowdhury and Fraser 2008). Al-Mathal (2008) were also noticed severe hyperplasia of the lining epithelium of the portal areas and fingerlike projections formatting in the lumen of the bile duct which is in consonance with the present study.

Oral administration of crude garlic ameliorated the adverse impacts of hepatic coccidiosis in rabbits when used as a prophylactic drug, but was less effective as a therapeutic agent (Abu-Akkada et al. 2010). Successful treatment of rabbit coccidiosis using sulphaquinoxaline was reported (Magray et al. 2010). Singla et al. (2000) reported that toltrazuril @ 25 ppm for 2 days in drinking water was highly effective for the treatment of clinical outbreaks of hepatic coccidiosis in rabbits. Sulphadimidine was used to treat adult coccidiosis in cattle (Reddy et al. 2013). Hygienic maintenance of rabbitry also plays an important role in control of the disease as the dampness of sheds favors the development of infective stages of coccidia which get transmitted by faecal oral route.

Conclusion

Impression smear examination is an old method when compare with the newly available methods like

histopathology techniques. But, literature availability and studies regarding the detailed impression smear staining was very little in Andhra Pradesh of India. This impression smear staining technique is simple, quick and good in clinical diagnosis of hepatic coccidiosis when compare with other diagnostic techniques.

Acknowledgements The authors acknowledge the authorities of Sri Venkateswara Veterinary University, Tirupati for providing facilities to carry out this research.

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