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Clinico-pathologic observations of spontaneous hepatic coccidiosis in broiler rabbits maintained in Bannerghatta biological park in Karnataka state of India

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ABSTRACT

The present study reports the clinico-pathologic changes observed in spontaneous hepatic coccidiosis caused by *Eimeria stiedae* in rabbits at Bannerghatta Biological Park (BBP) in Karnataka state of India. The clinical signs observed in the affected rabbits included sudden death, stunted growth, anorexia, weight loss, diarrhoea and abdominal pain. Increased liver enzymes and bilirubin was noticed upon biochemical examination. At necropsy, lesions included grossly enlarged liver (hepatomegaly) with presence of discrete yellowish-white nodules ranging from 1mm to 5mm size throughout the parenchyma containing a thick creamy white fluid. Faecal sample examination demonstrated the presence of *Eimeria* oocyts. The impression smears from the liver and intestinal mucosa also showed *Eimeria* oocysts, degenerative changes, necrotic cells and mononuclear cell aggregation to form oocyst granuloma. Histological observation revealed distension of bile duct, hyperplasia of epithelium along with numerous coccidian oocysts at the centre. Granulomatous tissues were observed to have encircled the bile duct with infiltration of inflammatory cells. Microscopic examination of exudates from liver also revealed numerous oocysts of *E. stiedae*. Multiple areas of coagulative necrosis of hepatic cells surrounded with inflammatory cells were also found.

Key words: *Eimeria stiedae*, Hepatic coccidiosis, Rabbit.

INTRODUCTION

Coccidiosis caused by an intestinal apicomplexan protozoan parasite of the genus *Eimeria,* is an economically important, highly contagious, fatal sporozoal infection seriously impairing the growth of infected animals and birds Pangasa *et al.,* 2007; Rehman *et al.,* 2011; Cavalcante *et al.,* 2012). It is one of the major protozoal diseases commonly observed worldwide in domestic rabbits (*Oryctolagus cuniculus*) (Wessels *et al.,* 2011) kept on the ground compared to those reared in cages. Rabbit coccidiosis has two anatomic forms: hepatic caused by *E. stiedae* and intestinal caused by other fourteen *Eimeria* species (Li and Ooi, 2009). Infection with hepatic coccidiosis is reported throughout the world (Satyanarayana *et al.,* 1982) and is economically more important since it reduces the carcass weight of rabbits by more than 23% (Barriga and Arnoni, 1981). *Eimeria stiedae* is a pathogenic organism and multiplies in the epithelial cells of bile ducts (Hauptman *et al.,* 2001; Al-Mathal, 2008). Though all age groups of rabbit are susceptible, young ones particularly housed in poor environmental sanitation and poor hygienic practices are more prone to hepatic coccidiosis (Erdogmus and Eroksuz, 2006; Al-Mathal, 2008; Gonzalez-Redondo *et al.,* 2008) and lead to death (Oncel *et al.,* 2011; Papeschi *et al.,* 2013). Polynucleate merozoites are characteristic of rabbit coccidia and so far this stage has not been found in other coccidian species. The gross lesion associated with hepatic coccidiosis is unique and nearly pathognomonic. Because *E. stiedae* causes proliferation of bile duct epithelial cells, affected livers contain multifocal, well-demarcated, linear, occasionally branching, bosselated, yellow to pearl-gray lesions that reflect the course of the biliary tree (Kim *et al.,* 2010). The coccidia of rabbits have not been studied to the same degree as the species, which occur in other hosts (Al-Mathal, 2008). In many parts of world there is a lack of information about this parasite and a few studies were reported regarding the parasitological aspect of the disease (Abbas, 2009). The aim of the present investigation was to document the clinico-pathological changes in spontaneously occurring hepatic coccidiosis in rabbits reared at Bannerghatta Biological Park (BBP), Karnataka, India. The results may improve the basic information on the current situation of the disease and for future research to develop the appropriate control strategy.

MATERIALS AND METHODS

Bannerghatta Biological Park maintains the broiler rabbit farm intended for feeding larger snakes in the zoo. The farm had 40 pair breeding stock of rabbits. Initially 3

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rabbits with rough hair coat with a history of diarrhoea and sudden death were submitted to the Wild Animal Disease Diagnostic Laboratory for necropsy. After necropsy, representative tissue specimens from the liver and intestine were collected and fixed in 10% neutral buffered formalin and processed for histopathological examination after staining with hematoxylin and eosin (H&E) as described earlier (Luna, 1968; El-Akabawy *et al.*, 2004). Each liver and gall bladder was removed and inspected for nodules typical of infection with *E. stiedae*. Impression smears were taken from the cut surface of the liver and the intestinal mucosa. The smears were dried, fixed with methanol and stained with the Giemsa stain and observed under the light microscope. The faecal samples were also collected and processed by sedimentation method for the microscopic examination (Gupta and Singla 2012; Manser *et al.*, 2016).

RESULTS AND DISCUSSION

On presentation, the carcasses were thin with reduced fat stores and muscle wasting. The hair coat was rough with adherent faecal material on the perineal hair. The main clinical signs observed in affected rabbits were depression, reduced appetite, brown watery diarrhoea, emaciation, abdominal pain, rough hair coat, pendulous and distended abdomen noted on abdominal palpation, pale mucous membranes, hepatomegaly, progressive weakness and death. These corroborate with earlier findings of Erdogmus and Eroksuz (2006) and Lakshmanan *et al.* (2011). Jaundice was also reported in two cases.

Clinical pathology: Serum biochemical examination revealed an elevated alkaline phosphatase and total bilirubin level. Serum glutamic oxaloacetate transaminase (aspartate aminotransferase) and serum glutamic pyruvate transaminase (alanine aminotransferase) activity was also increased. There was consistent bilirubinemia and transient hypoproteinaemia with hypoglycaemia (Sanyal and Sharma, 1990). Increased activity of these liver enzymes as well as bilirubinaemia is characteristic for the infection as reported previously (Hein and Lämmler; 1978, Barriga and Arnoni; 1981).

Gross pathology: Necropsy examination of rabbit revealed increased dirty dull straw colored fluid in the peritoneal cavity and enlarged liver with distended gall bladder. The size and weight of liver increased due to excessive proliferation and distension of bile duct epithelium, which is characteristic of this disease leading to pendulous and distended abdomen. These results agreed with Abbas (2009) and Singla *et al.* (2000). The caecum was enlarged and the serosa was congested (Fig 1A). The enlarged liver contained multiple 1 to 3 mm diameter, slightly raised, discrete to coalescing, yellowish white nodules scattered throughout the parenchyma (Fig 1B). These observations were in agreement with earlier reports (Magray *et al.*, 2010; Lakshmanan *et al.*, 2011). Grossly, the cut sections of liver showed thick yellowish creamy white cheesy contents within the nodules and the hepatic parenchyma was firm. The proliferated bile ducts cause damage to the liver parenchyma leading to post-necrotic scarring explaining the firm consistency of hepatic parenchyma. These results agreed with Singla *et al.* (2000) and Al-Mathal (2008) and these hepatic lesions might have led to increase in liver enzymes and bilirubin in serum (Raymond and Detrick, 2001).

Cytologic interpretation: The faecal samples collected and processed by sedimentation method for the microscopic examination again revealed the presence of *Eimeria* oocysts (Fig 2). The impression smears from cut surface of liver

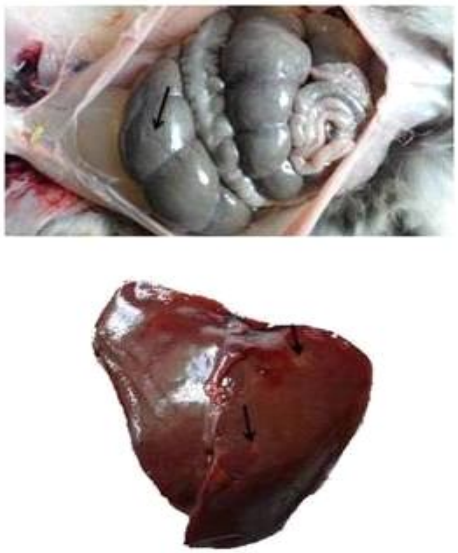

Fig 1: (A) Congested serosal membrane of the cecum; (B) Enlarged liver with yellowish- white nodules throughout the parenchyma shown by arrow marks.

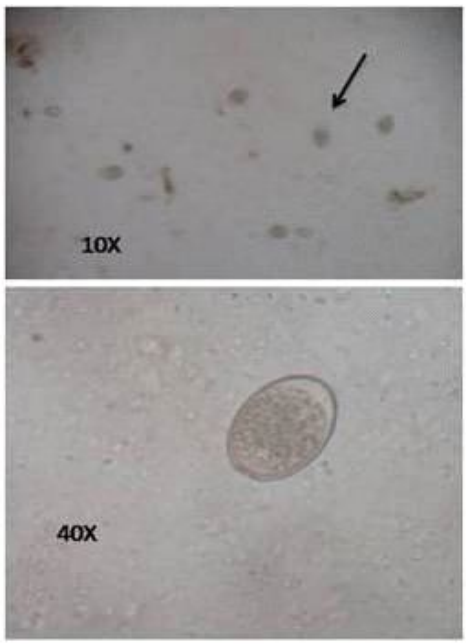

Fig 2: *Eimeria* oocysts observed in fecal sample examination by sedimentation method (10X and 40X).

showed the large number of unsporulated oocysts of *E. stiedae* intermixed with hepatobiliary parenchymal cells and numbers of inflammatory cells (Fig 3A and B). The oocysts were ovoid in shape with a micropyle and smooth yellowish orange coloured wall. Impression smears from the intestinal mucosa also revealed the presence of many oocysts and merozoites along with the inflammatory cells especially macrophages and necrotic cells debris. The findings corroborate with the study on impression smear of hepatic coccidiosis in rabbits earlier reported by Sivajothi *et al.* (2016).

Histopathology: The pathognomonic microscopic lesion was marked periportal fibrosis surrounding enlarged bile ducts (Fig 4) lined with hyperplastic bile duct epithelium (Fig 5) harboring inflammatory cell infiltrates. Fibrosis around the portal triad was also evident (Fig 6). *Eimeria stiedae* develops in the bile duct resulting in its hyperplasia (Pakandl, 2009). Kim *et al.* (2010) reported numerous oval *E. stiedae* oocysts in the convoluted hyperplastic bile ducts in the liver from a juvenile wild rabbit. The present histopathological observations i.e. bile ducts markedly enlarged and lined by hyperplastic columnar epithelial cells thrown into multiple arborizing papillary fronds extending into the ductal lumina with presence of developmental stages of *E. stiedae* and massive infiltration of lymphocytes, plasma cells and eosinophils resembled with those described by others (Toula, 2000; El-Akabawy *et al.*, 2004; Erdogmus and Eroksuz, 2006; Magray *et al.*, 2010; Lakshmanan *et al.*, 2011; Al-Naimi *et al.*, 2012).

Okumu *et al.* (2014) reported the prevalence, pathology and risk factors for coccidiosis in domestic rabbits in selected regions in Kenya. In France, 22.5% of dead broiler rabbits were infected with *E. stiedae* (Varga, 1982). In Brazil, 48% of rabbits that died in breeding farms and 64% of rabbits killed in abattoirs were infected with *E. stiedae* (Varga, 1982). Both clinical and subclinical form coccidiosis occured in domestic rabbits in Kenya and are major causes of diarrhea and death (Rashwan and Marai, 2000; Rosell *et al.*, 2010). Al-Mathal (2008) reported hepatic coccidiosis of the

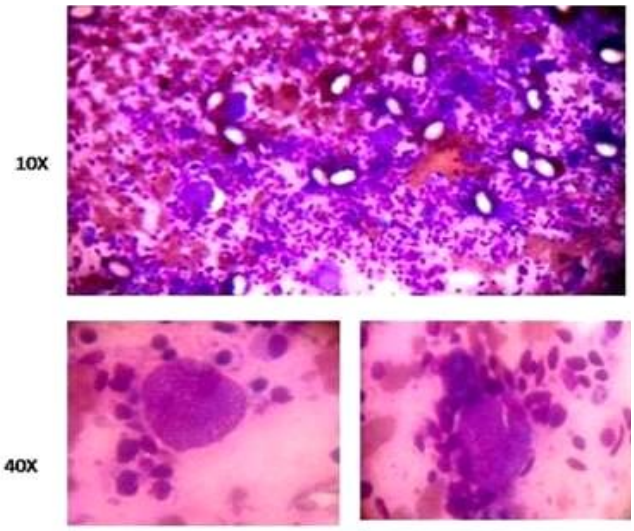

Fig 3(A): Liver impression smear from affected rabbit revealing numerous Eimerial oocysts, merozoites, many inflam matory cells and cellular debris by Giemsa staining (10X); (B) Liver impression smear showing the Eimerial merozoites, many inflammatory cells and cellular de bris by Giemsa staining (40X).

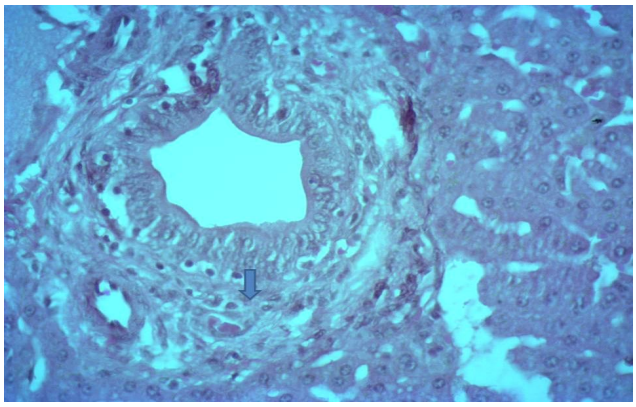

Fig 4: Fibrosis evident around the bile duct in liver of affected rabbit shown by arrow (40X).

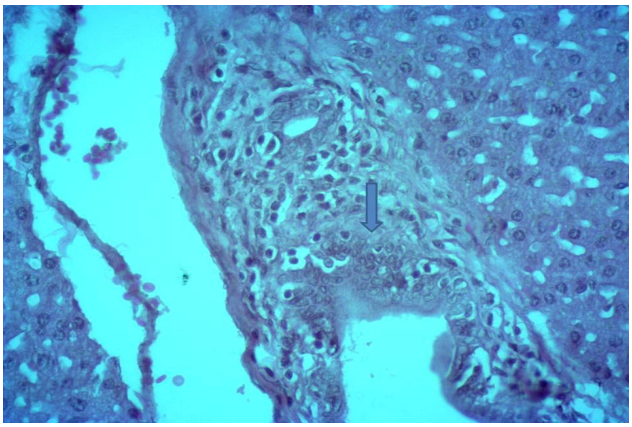

Fig 5: Arrow mark indicating bile duct lining cell hyperplasia in the liver of affected rabbit (40X).

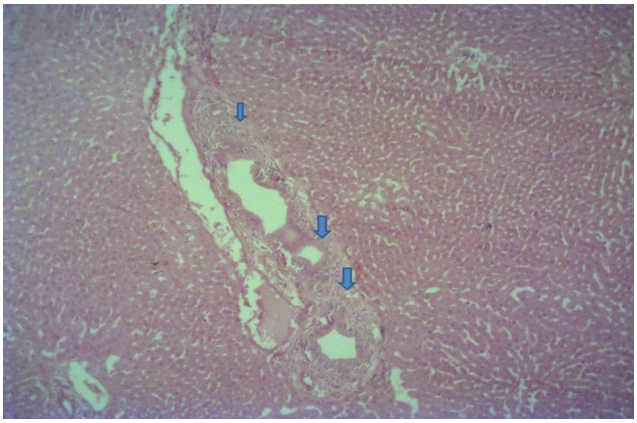

Fig 6: Microscopic liver section depicting fibrosis around the portal triad shown by arrow (10X).

domestic rabbit in Saudi Arabia. Hepatic coccidiosis in rabbits has also been reported from different parts of India such as Karnataka (D'Souza *et al.*, 1992), Himachal Pradesh (Jithendran and Bhat, 1995), Meghalaya (Rajkhowa, 1996; Laha *et al.*, 2015), Kashmir valley (Magray *et al.*, 2010), Kerala (Lakshmanan *et al.*, 2011) and Tamil Nadu (Palanivel *et al.*, 2013). Gavhane *et al.* (2015) reported hepatic coccidiosis with concurrent infection of pasteurellosis in rabbits in Mumbai region. Al-Naimi *et al.* (2012) reported pathological changes in hepatic coccidiosis in naturally infected rabbits. The management of severe hepatic coccidiosis in domestic rabbits in a rabbit farm in Kerala was reported by Becha and Devi (2014).

Maintaining a scrupulously clean, dry environment is the best way to prevent hepatic coccidiosis in rabbits. Food and water bowls should be cleaned regularly with mild disinfectants. If the bunny lives in a wire cage, the cage bottom must be brushed out every day with a brush that interrupts coccidian life cycle. Proper management and good programmed husbandry practices are crucial to reduce the rate of infection (Pakandl, 2009; Lakshmanan *et al*., 2011).

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REFERENCES

Abbas, S.M. (2009). Field study of some endoparasites in local rabbits. In: Proc. 9th Veterinary Scientific Conference, **1:** 157-160.

Al-Mathal, E.M. (2008). Hepatic coccidiosis of the domestic rabbit *Oryctolagus cuniculus domesticus* L. in Saudi Arabia. *World J. Zool.,* **3(1):** 30-35.

Al-Naimi, R.A.S., Khalaf, O.H., Tano, S.Y. and Al-Taee, E.H. (2012). Pathological study of hepatic coccidiosis in naturally infected rabbits. *AL-Qadisiya J. Vet. Med. Sci.,* **11(1):** 63-69.

Barriga, O.O. and Arnoni, J.V. (1981). Patho-physiology of hepatic coccidiosis in rabbits. *Vet. Parasitol.,* **8:** 201-210.

Becha, B.B. and Devi, S.S. (2014). Management of severe hepatic coccidiosis in domestic rabbits. *Indian J. Vet. Anim. Sci. Res.,* **43(1):** 44-48.

Cavalcante, A.C.R., Teixeira, M., Monteiro, J.P. and Lopes, C.W.G. (2012). *Eimeria* species in dairy goats in Brazil. *Vet. Parasitol.,* **183:** 356-358.

D'Souza, P.E., Rajeshwari, Y.B., Jagannath, M.S. and Rahman, S.A. (1992). Coccidiosis in rabbits. *Livestock Adv.,* **17:** 26-28.

El-Akabawy, L.M., Zayan, K.A., Tantawy, A.A. and Omar, R.E.M. (2004). Anticoccidial efficacy of propolis and Toltrazuril against *Eimeria stiedae* in New Zealand White Rabbit's. *Zagazig Vet. J.,* **32(1):** 129-145.

Erdogmus, Z.S. and Eroksuz, Y. (2006). DS hepatic coccidiosis in Angora rabbits. *J. Anim. Vet. Adv.,* **5(6):** 462-463.

Gavhane, D.S., Mhase, A.K., Moregaonkar, S.D., Sawale, G.K., Kadam, D.P. and Meshram, P.V. (2015). Hepatic coccidiosis with concurrent infection of pasteurellosis in rabbits. *Indian J. Vet. Pathol.,* **39(3):** 269-271.

Gonzalez-Redondo, P., Finzi, A., Negretti, P. and Micci, M. (2008). Incidence of coccidiosis in different rabbit keeping systems. *Arq. Bras. Med. Vet. Zootec.,* **60:** 1267-1270.

Gupta, S.K. and Singla, L.D. **(**2012) Diagnostic trends in parasitic diseases of animals. In: *Veterinary Diagnostics: Current Trends.* Gupta RP, Garg SR, Nehra V and Lather D (Eds), Satish Serial Publishing House, Delhi, pp 81-112.

Hauptman, K., Tichy, F. and Knotek, Z. (2001). Clinical diagnostics of hepathopathies in small mammals: Evaluation of importance of individual methods. *Acta. Vet. Brno.,* **70:** 297-311.

Hein, B. and Lämmmmler, G. (1978). Alteration of enzyme activities in serum of rabbits infected with *Eimeria stiedae*. *Parasitol. Res.,* **57:** 199-211.

Jithendran, K.P. and Bhat, T.K. (1995). Subclinical coccidiosis in Angora rabbits: A field survey in Himachal Pradesh. *World Rabbit Sci.,* **4:** 29-32.

Kim, D.Y., Reilly, T.J., Schommer, S.K. and Spagnoli, S.T. (2010). Rabbit tularemia and hepatic coccidiosis in wild rabbit. *Emerg. Infect. Dis.,* **16(12):** 2016-2017.

Laha, R., Das, M. and Goswami, A. (2015). Coccidiosis in rabbits in a subtropical hilly region. *Indian J. Anim. Res.,* **9(2):** 231-233.

Lakshmanan, B., Ravindran, R., Vasudeven, V.N. and Devada, K. (2011). Hepatic coccidiosis in rabbits in Kerala. *J. Ind. Vet. Assoc.,* **9(2):** 56-57.

Li, M. and Ooi, H. (2009). Fecal occult blood manifestation of intestinal *Eimeria* spp. infection in rabbit. *Vet. Parasitol.,* **161:** 327-329.

Luna, L.G. (1968). Manual of Histological Staining Methods of the Armed Forces Institute of pathology. 3rd. ed Mcgrow-Hill book company New York.

Magray, A.A., Khan, A.R. and Rasheed, M. (2010). Study on fatal hepatic coccidiosis in rabbits of Kashmir Valley. *J. Env. Bio. Sci.,* **24:** 243-245.

Manser, M.M., Santos Saez, A.C. and Chiodini, P.L. (2016). Faecal parasitology: concentration methodology needs to be better standardised. *PLoS Negl Trop Dis.,* **10(4):** e0004579.

Okumu, P.O., Gathumbi, P.K., Karanja, D.N., Mande, J.D., Wanyoike, M.M., Gachuiri, C.K., Kiarie, N., Mwanza, R.N. and Borter, D.K. (2014). Prevalence, pathology and risk factors for coccidiosis in domestic rabbits (*Oryctolagus cuniculus*) in selected regions in Kenya. *Vet. Quart.,* **34(4):** 205-210.

Oncel, T., Gulegen, E., Senlik, B. and Bakirci, S. (2011). Intestinal coccidiosis in Angora rabbits (*Oryctolagus Cuniculus*) caused by *Eimeria intestinalis, Eimeria perforans* and *Eimeria coecicola*. *Yüzüncü Yýl Üniversitesi Veteriner Fakültesi Dergisi.,* **22(1):** 27-29.

Pakandl, M. (2009). Coccidia of rabbit: a review. *Folia Parasit.,* **563:** 153-166.

Palanivel, K.M., Sureshkumar, K. and Rishikesavan, R. (2013). Prevalence of hepatic coccidiosis in rabbits - A postmortem analysis. *Indian Vet. J.,* **90(11):** 72-73.

Pangasa, A., Singla, L.D., Sood, N., Singh, A. and Juyal, P.D. (2007). Histopathological evaluation of anticoccidial activity of an ayurvedic coccidiostat, in induced *Eimeria tenella* infection in chicken. *Indian J. Anim. Sci.* **77**(3): 214-216.

Papeschi, C., Fichi, G. and Perrucci, S. (2013). Oocyst excretion pattern of three intestinal *Eimeria* species in female rabbits. *World Rabbit Sci.,* **21:** 77-83.

Rajkhowa, C. (1996). Incidence of rabbit coccidiosis in Meghalaya and its control. *J. Vet. Parasitol.,* **10:** 175-178.

Rashwan, A.A. and Marai, I.F.M. (2000). Mortality in young rabbits: a review. *World Rabbit Sci.,* **8:** 111-124.

Raymond, J.L. and Detrick, F. (2001). Pathology of rabbits. U.S Army Medical Research Institute of Infectious Disease. Raymonde det. Amedd. Army. Mil.

Rehman, T.U., Khan, M.N., Khan, I.A. and Ahmad, M. (2011). Epidemiology and economic benefits of treating goat coccidiosis. *Pakistan Vet. J.,* **31(3):** 227-230.

Rosell, J.M., de la Fuente, L.F., Badiola, J.I., Fernandez de Luco, D., Casal, J. and Saco, M. (2010). Study of urgent visits to commercial rabbit farms in Spain and Portugal during 1997-2007. *World Rabbit Sci.,* **17:** 101-127.

Sanyal, P.K. and Sharma, S.C. (1990). Clinicopathology of hepatic coccidiosis in rabbits. *Indian J. Anim. Sci.,* **60:** 924-928.

Satyanarayana, N.V.V., Madhekar, D. and Rao, C.R.S. (1982). Observations on an outbreak of coccidiosis in rabbits and its treatment. *Livestock Adv.,* **7:** 1-57.

Singla, L.D., Juyal, P.D. and Sandhu, B.S. (2000). Pathology and therapy in naturally *Eimeria stiedae* infected rabbits. *J. Protozool. Res.,* **10:** 185-191.

Sivajothi, S., Reddy, B.S. and Rayulu, V.C. (2016). Study on impression smears of hepatic coccidiosis in rabbits. *J. Parasitic Dis.,* **40(3):** 906-909.

Toula, F.H. (2000). Efficacy of water Suspension of Dried leaves of neem (*Azadirachta indica*) in the Control of hepatic coccidiosis in Rabbit (*Oryctolagus Cuniculus*) in Jeddah (Western Region of Saudi Arabia). *J. Egyptian-German Soc. Zool.,* **33:** 107-128.

Varga, I. (1982). Large-scale management systems and parasite populations: coccidia in rabbits. *Vet. Parasitol.,* **11:** 69-84.

Wessels, J., Wessels, M.E., Wood, R. and Quayle, J. (2011). Hepatic coccidiosis in red lechwe (*Kobus leche leche*). *Vet. Rec.,* **168:** 338.