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SHORT COMMUNICATION

Occurrence of Necrotic Enteritis associated with Coccidiosis in a Desi Chicken (*Gallus gallus domesticus*) flock

Prajakta Dethe, V.S. Dhaygude*, M.S. Budhe[1], Chandan H.U., Surabhi Bhandekar and U.M. Tumlam[1]
Department of Veterinary Pathology, K.N.P. College of Veterinary Science, Shirwal, Maharashtra Animal and Fishery Sciences University, Nagpur; [1]Department of Veterinary Microbiology

Address for Correspondence:
V.S. Dhaygude, Department of Veterinary Pathology, K.N.P. College of Veterinary Science, Shirwal, Maharashtra Animal and Fishery Sciences University, Nagpur; E-mail: vithaldhaygude@mafsu.ac.in

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ABSTRACT

The present investigation describes the occurrence of necrotic enteritis and a concurrent intestinal coccidiosis in a desi chicken (*Gallus gallus domesticus*) flock (Size:-1400). Necrotic enteritis is an acute enterotoxaemia caused by *Clostridium perfringens;* a quite ubiquitous bacterium readily found in soil, dust, faeces, feed and used poultry litter. It is considered as a normal inhabitant of the intestine of healthy chickens. Along with many other factors, the coccidiosis predisposes birds to necrotic enteritis. The 5 carcasses of 137 days old desi chicken were presented to the Department of Veterinary Pathology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Dist. Satara for the necropsy with the history of dullness, inappetence and mortality. Gross examination revealed diffuse congestion of small intestine. On opening severe necrosis of mucosa of small intestine along with foul-smelling reddish-brown contents in the lumen were observed. Mucosa had diphtheritic appearance due to yellowish material covering it. Multifocal haemorrhages were also noted. The wet mount preparation of intestinal content revealed presence of oocysts of *Eimeria spp*. The impression smear from jejunum stained with Grams Stain showed large number of Gram-positive rod-shaped bacteria. Representative samples were collected for histopathological and molecular diagnosis. Histopathological examination showed severe necrosis of the epithelial cells lining the small intestine, fusion of villi and severe infiltration with polymorphonuclear cells, presence of oocysts and other developmental stages of *Eimeria spp.* The PCR amplification of *16s rRNA* gene of *Cl. perfringens* species and subsequent agarose gel electrophoresis of PCR product revealed bands of 481 bp to confirm the infection. The gross and microscopic lesions, microscopic examination of intestinal contents and PCR confirmed the necrotic enteritis in the desi chicken flock which is of economic and public health significance.

Key words: Necrotic enteritis, coccidiosis, gross & histopathological lesions, PCR

Necrotic enteritis has devasting economic effects on production due to high mortality rates and poor feed efficiency. *Clostridium* spp. are considered to be one of the most important agents causing enteric diseases in poultry. Diagnosis of enteric diseases produced by Clostridia is usually challenging mainly because many clostridial species can be normal inhabitant of gut[1], making it difficult to determine their role in virulence. Most common clostridial enteric disease in poultry is necrotic enteritis caused by *Clostridium perfringens* Type A and to lesser extent Type C.[2] *Clostridium* is Gram positive, rod shaped, spore forming and anaerobic bacteria. Necrotic enteritis develops when *Cl perfringens* multiplies anaerobically in the chicken intestinal tract, producing toxins that leads to necrosis. In healthy chicken *Cl. perfringens* is present at level of less than 10^5cfu/g intestinal content.[3] Most important known predisposing factor for necrotic enteritis, is intestinal damage caused by coccidial pathogens.[4] Intestinal damage will result in release of plasma proteins into lumen of intestinal tract. Leaking plasma of intestinal lumen can provide a necessary growth substrate for extensive proliferation of these bacteria to cause necrotic enteritis. The association between *Emeria spp.* and *Cl.perfringens* leads to necrotic enteritis[5] and has been recorded in India also. However, there are very few reports regarding the same in India, hence reported.

The 5 carcasses of 137 days old desi chicken with history of clinical signs *viz.* dullness, inappetence and sudden mortality were presented to the Department of Veterinary Pathology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Dist. Satara for the necropsy and subsequent diagnosis. Through necropsies were conducted to record gross lesions in various organ systems. The impression smears from affected intestine were prepared and stained with Gram's stain. Wet mount examination of intestinal content was also done. The parts of affected intestine were collected for histopathological examination and fixed in 10% neutral buffered formalin. After fixation, tissues were processed by paraffin embedding technique, blocks

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were prepared and sections were cut at 5µm thickness with a rotatory microtome and stained with routine Haematoxylin and Eosin staining method. Swabs from intestinal content were collected and inoculated in Robertsons cooked meat broth. It was incubated at 37°C for 24 hrs in an anaerobic condition and subsequently cultured on TSC (Tryptose-Sulfite-Cycloserine) agar.

The DNA was extracted from fresh colonies by snap chill method of DNA extraction. For molecular detection of the organism the PCR targeting *16s rRNA* gene was done with the forward (TAACCTGCCTCATAGAGT) and reverse (TTTCACATCCCACTTAATC) primers. The PCR conditions for 30 amplification cycles included denaturation at 94°C for 1 min, annealing at 55ºC for 30 sec, extension at 72°C for 90 sec and final extension at 72°C for 4 min. PCR product was confirmed by agarose gel electrophoresis.

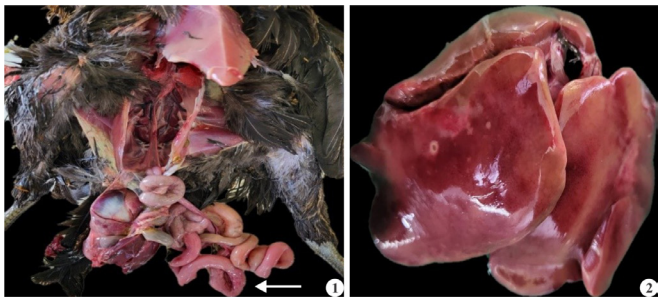

Fig.1. Congested loops of small intestine (arrow); **Fig.2.** Multifocal, pale strips with focal areas of necrosis and haemorrhages on liver

The gross examination revealed diffusely dark red (congested) intestinal loops (Fig.1) along with distended lumen due to accumulation of gas. Cut section of intestinal loop showed presence of foul smelling dark brownish fluid. Mucosal surface of intestine was covered with diphtheritic pseudomembranous made of fibrononecrotic material and mucosa had typical dirty turkish towel like appearance (Fig.3 and 4). Multifocal, pale white strips/ roundish areas of necrosis and haemorrhages were observed on liver (Fig.2). The microscopic examination of section of small intestine showed severe diffusely necrosed mucosa with fusion of villi, loss of epithelial

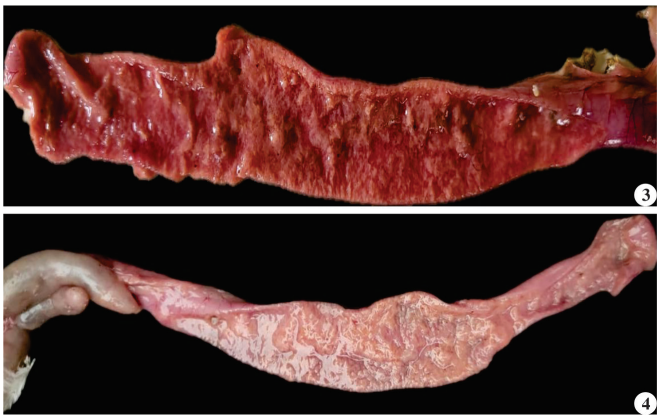

Fig.3. Severe diffuse necrotizing and haemorrhagic enteritis (Small intestine) with focal areas of mucosal sloughing. Mucosa revealed typical dirty turkish towel like appearance; **Fig.4:** Typical dirty turkish towel like appearance of the mucosa of intestine

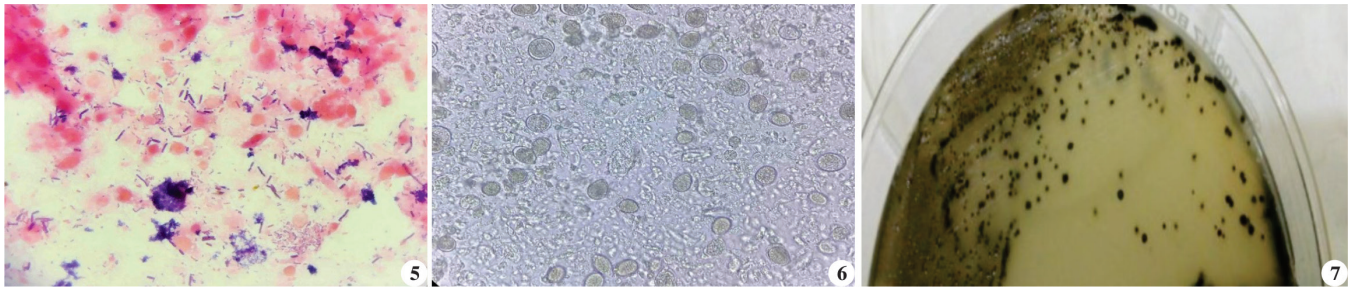

Fig.5. Typical Gram positive rods of *Clostridium spp.*in impression smear from jejunum (Gram stain, 100X); **Fig.6.** Oocysts of *Eimeria* spp. (400X); **Fig.7.** Black colonies of *Cl.perfringens* on clostridial agar with TSC supplement

cells and microvilli. Severe diffuse infiltration of polymorphonuclear cells predominantly heterophils in mucosa and submucosa was also a characteristic finding. There was sloughing of epithelial cells. Focally dark bluish purple colonies of *Cl. perfringens* were also noted. Also, round/oval oocysts and schizonts of *Eimeria* spp. were noted in mucosa (Fig.8 and 9).

The wet mount preparation of intestinal content revealed presence of oocysts of *Eimeria* spp. (Fig.5). Impression smear from jejunum stained with Gram stain revealed large number of Gram-positive rod-shaped bacteria (Fig.6). The bacterial growth on Robertsons Cooked Meat broth was characterized by production of acid and gas (without digestion of meat). On TSC agar typical black colonies were produced due to reduction of sulphite to ferrous sulphate (Fig.7) which indicated successful cultivation of *Cl perfringens*.

The DNA was successfully extracted from the bacteria colonies and the PCR amplification of *16s rRNA* gene of *Cl perfringens* yielded bands *of* expected 481 bp size confirming the involvement of *Cl. Perfingens* in necrotic enteritis (Fig.10).

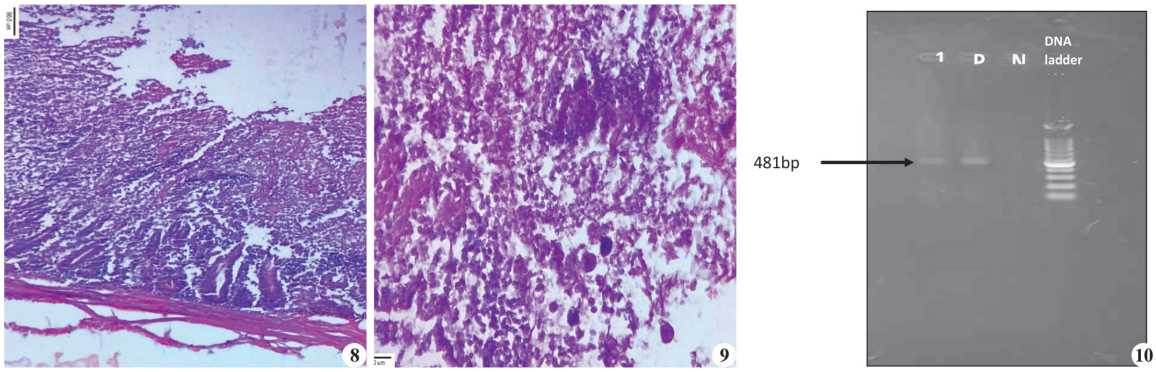

Fig.8. Severe and diffuse necrosed mucosa with fusion of villi, loss of epithelial cells and microvilli, severe infiltration of polymorphonuclear cells and focally dark bluish purple colonies of *Cl.perfringens* in the mucosa (H and E Stain ,100X); **Fig.9.** Section of intestine showing severe necrosis, bacterial colonies, oocysts and other development stages of *Eimeria* spp. (H and E Stain,400X); **Fig.10.** Result of PCR assay showing amplification of 481 bp specific for *Cl perfringenes* (Lane L : 100 bp DNA ladder, 1 : sample, P : Positive control, N : Negative control)

Necrotic enteritis is one of the major enteric diseases caused by *Cl perfringens* mostly Type A and Type C in rare cases. There are many predisposing factors, like damage to intestinal epithelium, disturbance in gut microbial composition, immunosuppression, etc. But main predisposing factor is coccidial infection caused by *Eimeria spp*[6]. Damage to intestine due to coccidia results in inflammatory response and release of plasma proteins into lumen of intestine. These plasma proteins provide necessary growth substrate for proliferation of these bacteria[5]. *Cl perfringens* multiplies anaerobically in intestinal tract and produces toxin. These toxins are proteolytic and collagenolytic enzymes that damage intestinal epithelium and causes necrosis. Gross lesions such as diffusely congested intestinal loop with fibrononecrotic material in lumen was noted and recorded earlier[8-10]. Histopathological changes including diffusely necrosed mucosa, sloughed off epithelial cells, infiltration of inflammatory cells and presence of coccidial oocysts and schizonts were noted and also reported earlier[8-11]. *Cl. perfringens* goes to liver through portal bloodstream and biliary duct and causes damage to liver.[7] Gross lesions such as necrotic foci and heamorrhages were observed and same has been recorded on the present investigations also[9,10].Histopathologically loss of hepatocytes, sinusoidal fibrosis, congestion and infiltration of inflammatory cells has been recorded earlier[7,12]. The association between *Emeria spp.* and *Cl.perfringens* leads to necrotic enteritis[5] and has been recorded in India also. However, the records regarding the same are scant, hence reported.

Based on characteristic gross lesions, wet mount examination and bacterial examination of intestinal content, histopathological findings and PCR detection of 16S rRNA gene; the case was confirmed as necrotic enteritis and concurrent coccidiosis.

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