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Short Communication

Clostridium perfringens Type D Enterotoxaemia in the Chinkara Deer (*Gazella bennettii*)

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Abstract: During an enterotoxaemia outbreak, 9 out of 11 (82%) Chinkara deer (*Gazella bennettii*) died; 5 animals died of peracute disease, whereas acute death was observed in 4 animals. The case fatality rate was 100%. Clinical signs, including high temperature, depression, anorexia, abdominal pain, greenish watery diarrhoea, and respiratory distress, were recorded in the affected deer. Post-mortem examinations of the affected animals revealed muscular and serosal haemorrhages, hydroperitoneum, hydrothorax, and hydropericardium. Severe haemorrhages were also observed in the small intestine, colon, and kidneys. Histopathologically, haemorrhagic enterocolitis, pulmonary oedema, accumulation of proteinaceous fluid in alveoli, perivascular oedema, and interstitial and intertubular haemorrhages were seen in the kidneys. Anaerobic incubation, mouse inoculation, and mouse seroneutralisation with intestinal contents collected from the affected deer confirmed the Chinkara deer were infected with *Clostridium perfringens* type D.

Key Words: Chinkara deer, enterotoxaemia, *Clostridium perfringens* type D, clinical picture, pathological lesions

Enterotoxaemia is an acute toxaemia caused by the proliferation of the gram-positive anaerobe, *C. perfringens* type D, in the small intestine and the liberation of epsilon toxin (1,2), and causes severe vascular damage (3). Spores of the causative organism are found in soil and in faeces of healthy animals raised in areas where the disease is prevalent. In healthy animals, most of the ingested *C. perfringens* type D are destroyed in the rumen and abomasum. Although the alkaline pH of the duodenum is quite favourable for multiplication of these bacteria, toxaemia does not occur, as continual movement of ingesta keeps the bacterial population and toxin contents low. Animals with high levels of epsilon toxin may move about without showing signs of illness until found dead or exhibiting the acute form of enterotoxaemia (4).

The disease is prevalent in sheep and goats, with peracute cases occurring at 3-10 weeks of age (5), although both acute and chronic enterotoxaemia can occur in both young and adult sheep and goats. The tendency for chronic cases to occur is relatively higher in vaccinated adult goats, while acute enterotoxaemia usually occurs in unvaccinated young and adult goats; however, this disease has not been previously reported in the Chinkara deer (*Gazella bennettii*). The present manuscript describes the clinical picture and bacterial isolation, as well as gross and histopathological lesions in 11 Chinkara deer with enterotoxaemia.

The present study included 11 clinically healthy Chinkara deer (4 stags, 3 hinds, and 4 fawns) maintained at a private farm in Faisalabad, Pakistan. The stags and hinds were fed green fodder (alfalfa) and grams (a protein-rich grain), while the fawns were fed mostly dam's milk and were occasionally offered green fodder. These animals were not vaccinated against any disease; however, de-worming was carried out 4 times a year. Affected animals were treated with injectable tylosin tartrate (Tylosan-20®, Sana Laboratories, Faisalabad, Pakistan) 10 mg kg^{-1} of body weight IM and dexamethasone (Star Labs, Lahore, Pakistan) 1 mg kg^{-1} of body weight IV daily. Despite 4-5 days of treatment, none of the affected animals could be cured.

Post-mortem examinations were conducted within 30 min of death and morbid tissues were collected and fixed

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in 10% buffered formalin. Tissue sections 5 µm thick were cut and processed for histopathological studies using the routine haematoxylin and eosin (H&E) staining technique. Faecal samples were collected from dead animals and examined microscopically for parasitic infestation using the floatation technique (6). For bacteriological analysis, a loopful of ingesta from the small intestine was immediately inoculated onto blood agar and incubated aerobically, as well as anaerobically, at 40 ºC. After 48 h of incubation, the colonies with the characteristic double halo of haemolysis were re-streaked onto cooked meat medium, egg yolk agar, and gelatin stab, and incubated at 37 ºC under anaerobic conditions for 48 h.

Samples of intestinal contents were also centrifuged at 32,000 × g at 4 ºC for 40 min. The supernatant was divided into 2 equal parts; one part was kept as is, while the second part was incubated at 37 ºC for 1 h after adding 1% trypsin (Difco, USA). Then, 0.2 ml of each part was inoculated intraperitoneally into 2 mice that were kept under close observation for 2 days. Another group of 2 mice was inoculated with the supernatant mixed with antisera against *C. perfringens* types A, B, C, or D (Sigma-Aldrich, USA), according to the manufacturer's recommendations.

Due to this outbreak mortality reached 82% (9 out of 11 animals died). Among the deaths, 56% were the result of peracute disease and the remaining 44% were cases of acute onset. The case fatality rate was 100%. None of the animals that died from peracute disease showed any clinical signs or symptoms; however, the acute cases were off feed and suffered from restlessness, abdominal pain, salivation, high temperature (40.5 °C), severe respiratory distress, and greenish watery diarrhoea. In the advanced stages of disease staggering gait, dehydration, emaciation, and death occurred.

Upon microscopic examination, faecal samples were negative for endoparasites. No growth of aerobic or anaerobic micro-organisms of pathogenic importance was observed, except gram-positive short and thick rods. Anaerobic organisms caused partial to complete haemolysis of the blood agar, liquefied gelatin medium, turned cooked meat medium to pink, and were positive for Nagler's reaction (opalescent on egg yolk agar). A toxin lethal for mice was detected in the supernatant of the trypsinised intestinal contents collected from affected deer, which was identified as epsilon by the antiserum neutralisation test. Confirmatory diagnosis of enterotoxaemia caused by *C. perfringens* type D in deer requires identification of typical bacteria or toxins in intestinal contents (7). Laboratory findings of the present study were suggestive of *C. perfringens* type D infection in the affected Chinkara deer.

Post-mortem examination of the affected deer revealed pinpoint haemorrhages on all serosal surfaces and in the muscles. The lumen of the small intestine and colon contained greenish watery fibrino-necrotic material. Body cavities contained increased amounts of straw-coloured fluid, indicating hydroperitoneum, hydrothorax, and hydropericardium. Severe haemorrhages on the mucosa of the abomasum, small intestine, and myocardium were also observed. Severe haemorrhagic, reddish black and soft/pulpy kidneys were a consistent feature. Lungs were consolidated, congested, and haemorrhagic, and contained oedematous fluid.

Histopathologically, there was haemorrhagic enteritis and enterocolitis, and the intestinal lumen contained fibrino-necrotic material comprised of desquamated cells, fibrin, leukocytes, tissue debris, etc. Intestinal villi were desquamated, congested, eroded, and necrotic. There was infiltration of polymorphs in the muscularis mucosae, which in most of the cases was not intact. Abomasal mucosa was congested and haemorrhagic. Pulmonary oedema was a consistent feature and the alveoli were filled with proteinaceous fluid, infiltrated with polymorphs, and had broken interalveolar septa (Figure 1). Another characteristic feature recorded was engorged blood vessels with erythrocytes and thickened alveolar septa, along with perivascular oedema. In the affected bronchioles, lining epithelium was detached and degenerative changes were obvious. The walls of these bronchioles were thickened and extensively infiltrated by inflammatory cells (Figure 2).

Kidneys showed severe congestion, with pyknotic nuclei and necrosis of renal tubules, intertubular and interstitial haemorrhages, and oedematous fluid (Figures 3 and 4). Epithelium of the affected renal tubules was sloughed off into the lumen (Figure 4). In the glomeruli, tuft capillaries were shrunken, with increased urinary spaces. Severe congestion and oedema of the renal medullae was also observed.

In the accessible literature, enterotoxaemia caused by *C. perfringens* type D has been reported in fallow deer (8), reindeer (9), and young red deer (10); nonetheless,

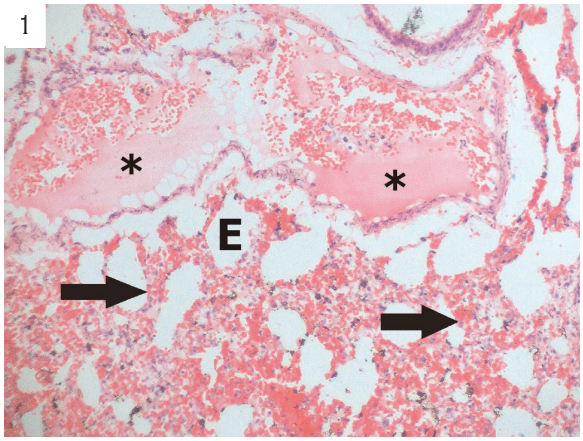

Figure 1. Photomicrograph of the lungs of a deer that died due to enterotoxaemia shows thickened (arrows) and congested alveolar walls, emphysema (E), and oedematous fluid (*) (H&E, 100×).

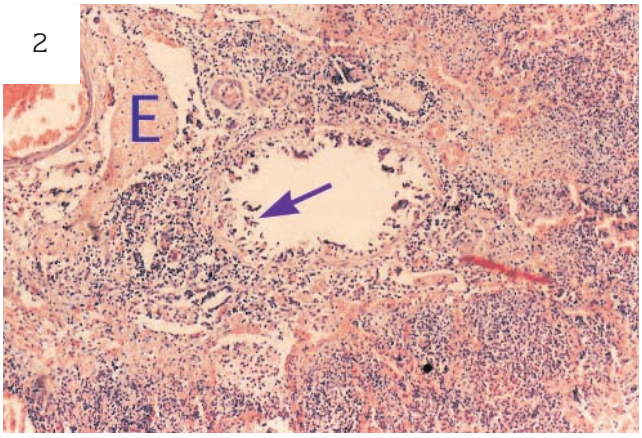

Figure 2. Photomicrograph of a bronchiole of a deer that died due to enterotoxaemia shows desquamation of epithelium (arrow), extensive peri-bronchiolar infiltration, and accumulation of oedematous fluid mixed with fibrin (E) (H&E, 100×).

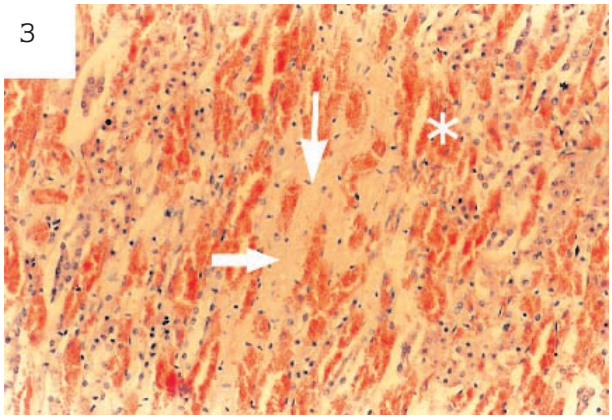

Figure 3. Photomicrograph of the kidney of a deer that died due to enterotoxaemia shows extensive congestion (*), necrosis, and oedematous fluid accumulation (arrows) in the renal tubules (H&E, 100×).

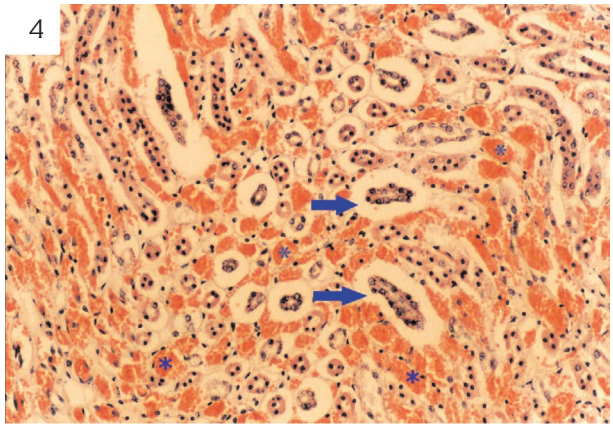

Figure 4. Photomicrograph of the kidney of a deer that died due to enterotoxaemia shows severe congestion (*), haemorrhages, and pyknotic nuclei (necrosis) of the renal tubules, along with sloughing of tubular epithelium (arrows) (H&E, 100×).

this disease is not common in deer (7). To the best of our knowledge the present study is the first to report enterotoxaemia caused by *C. perfringens* type D in Chinkara deer in Pakistan.

Signs and symptoms of enterotoxaemia remain mostly unnoticed due to the peracute or acute nature of the disease (1), and affected deer may be found dead, or may die in convulsion within 24 h after showing signs of anorexia, diarrhoea, and depression (7). In the present study most of the deer (56%) did not exhibit any clinical sign. Diarrhoea is reported to be a cardinal clinical sign of acute and chronic forms of enterotoxaemia in goats (5). In acute cases marked abdominal pain/discomfort, profuse watery diarrhoea with faeces containing fibrin clots, irregular rapid respiration, various nervous signs (motor in-coordination, severe colonic convulsion with frothing at mouth), and rapid death have been reported. Affected animals that survive for a few hours exhibit a staggering gait, opisthotonos, recumbency, severe convulsions, and death (1).

C. perfringens type D is a commensal organism in soil and the intestinal tract of sheep and goats (5), and of fallow, reindeer, and young red deer (8-10). The acidic environment of the abomasum, peristalsis, and limited quantities of fermentable substrates in the intestinal tract prevent rapid growth of the organisms under

physiological conditions; however, multiplication of bacteria and production of toxins are enhanced by a diet rich in carbohydrates and proteins (1,5), a sudden change in diet (11), extensive grazing on lush green fodder, heavy worm infestation, and environmental stress (1,5). In the present study deer were fed green fodder, i.e. alfalfa. Moreover, change of weather from hot and dry to hot and humid also caused stress for the animals. In the intestine, a reduction of peristaltic movements could have occurred due to a diet rich in carbohydrates and proteins (grams), and fodder low in fibre, but high in water content. Reduced peristalses could have provided an ideal environment for bacterial proliferation and liberation of toxins in these animals.

The clinical signs, pathological findings, isolation of *C. perfringens* type D in pure culture from intestinal contents, and demonstration of epsilon toxin in the gut contents led to the diagnosis of enterotoxaemia in these animals. Major toxins released by the organism include epsilon (12,13) and phospholipase C (13). Epsilon is a prototoxin and is activated by proteolytic enzymes. It enhances the permeability of the intestine and increases its own absorption (5). After absorption this toxin causes widespread vascular damage and increases vascular permeability, leading to degeneration of vascular endothelium, perivascular oedema, and accumulation of protein-rich fluid in the heart and lungs (14). Excessive accumulation of clear straw-coloured fluid in the pericardial sac in young red and fallow deer (7), and reddish gelatinous fluid in the abdominal and pericardial cavities in sheep that died due to enterotoxaemia (12) have been reported. Pulpy/soft kidneys are an important post-mortem finding in sheep, goats (15), and deer (7) that die due to enterotoxaemia. Hydroperitoneum, hydrothorax and hydropericardium, pulmonary and perivascular oedema, haemorrhagic enterocolitis, along with haemorrhagic lesions in nearly all organs are the consistent features in affected sheep and goats (15). The greenish watery contents in the intestine and haemorrhages in the myocardium observed in the present study have also been reported in deer that died due to enterotoxaemia (7).

It was concluded from the present study that enterotoxaemia can occur in Chinkara deer with clinical signs and pathological lesions similar to those of other small ruminants. Epsilon is the principal toxin responsible for the lesions.

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