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Pathological study of kidneys in *Clostridium perfringens* type D enterotoxemia in sheep

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Abstract
Type D enterotoxemia is a highly fatal enterotoxemia also known as pulpy kidney disease which causes heavy economic losses to sheep industries. It is caused by toxins secreted from *Clostridium perfringens* type D organism a gram positive, spore forming, and an obligate anaerobic rod bacterium. In the present investigation, a detailed necropsy was performed on 362 sheep irrespective of age, sex and breeds. Out of these, 123 sheep suspected for enterotoxemia were processed for molecular confirmation of *Clostridium perfringens* type D from intestinal contents by using PCR. After conducting PCR, 66 sheep found positive for *Clostridium perfringens* type D which was processed for histopathological examination. An overall incidence of enterotoxemia infection in sheep was recorded as 18.23 per cent.. Grossly, kidneys were swollen, darkly congested, and appeared soft and pulpy in consistency. The pulpy kidneys were brownish yellow to a distinct yellow in colour. Microscopically, kidneys showed cloudy swelling, coagulation necrosis, haemorrhages, interstitial nephritis along with infiltration of polymorpho and mononuclear cells, and vascular congestion.

Keywords: sheep, histopathology, kidney, enterotoxemia

Introduction
Enterotoxemia or pulpy kidney disease is caused by *Clostridium perfringens* type D [4] which normally presents in the intestine of ruminants. (1) *Clostridium perfringens* type D produces alpha and epsilon toxins [3, 5]. It is a highly lethal disease of sheep, goat and other animal species [12]. However, predominant in sheep and goats with per acute cases occurring at 3-10 weeks of age [18]. It both acute and chronic cases can found in both young and adult sheep. Predisposing factors that change the intestinal environment, such as high levels of carbohydrates, high-protein diets and grasses, can increase the numbers of *Clostridium perfringens*, along with toxin production [16]. Moreover, over-consumption of large amounts of milk, inadequate colostrum intake, chilling and stress are also favourable conditions in order to development of this disease. A sudden change in the diet is predominantly associated with this disease [6]. Kidney is one of the target organs of epsilon toxin. Therefore, it is called pulpy kidney. It is a significant in terms of diagnosis in ovine enterotoxaemia [19]. It is believed to be a post-mortem change rather an ante mortem change [17]. Diagnosis is made by gross and histopathological examinations [15].

Material and methods
The affected tissue samples of lungs, liver, kidneys, heart, spleen, mesenteric lymph nodes, intestine, rumen and abomasums from carcasses of sheep were collected for proposed investigation irrespective of sex, age groups and breeds from various Veterinary hospitals, rural areas in and around Bikaner district of Rajasthan.
The samples received from field veterinarians in the Department of Veterinary Pathology were also included in this study.
Necropsy was performed on 362 sheep irrespective of age, sex and breeds. Out of these, tissue samples from 123 sheep suspected for enterotoxemia were further preserved in 10 percent formalin. The parts of affected tissue measured 2-5 mm thickness and presenting the lesions with normal tissue, were used for fixation and histopathological examination.
For histopathological examination, processing of tissue was done by paraffin embedding using acetone and benzene technique (Lillie, 1965). The tissue sections of 4-6 micron thickness were cut and stained with hematoxylin and eosin staining method as a routine.

Results

Necropsy was performed on 362 sheep irrespective of age, sex and breeds. Out of these, 123 sheep suspected for enterotoxemia were processed for molecular confirmation of *Clostridium perfringens* type D from intestinal contents by using PCR. After conducting PCR, 66 sheep found positive for *Clostridium perfringens* type D which was further processed for histopathological examination. An overall incidence of enterotoxemia infection in sheep was recorded as 18.23 percent.

Grossly

Kidneys were swollen, darkly congested and appeared soft and pulpy in consistency (Fig.1). The pulpy kidney was brownish yellow to a distinct yellow in colour.

Microscopically

Glomerular necrosis was found in 32 cases (48.48%). Renal tubular necrosis was found in 49 cases (74.24%). Fatty change was observed in 2 cases (3.03%). Congestion and oedema was found in 39 cases (59.09%). Haemorrhages were found in 45 cases (68.18%). Inflammatory cells infiltrations were observed in 63 cases (94.45%). Vacuolar degeneration was observed in 6 cases (9.09%), protein cast in 1 case (1.51%), and cloudy swelling observed in 7 cases (10.60%).

Kidneys revealed congestion and intertubular haemorrhages in renal medulla. Some cases showed massive intratubular haemorrhages and intertubular haemorrhages along with glomerular haemorrhage and edema. Kidneys showed interstitial nephritis in which infiltration of mononuclear cells and polymorphonuclear cells in interstitium, and intratubular haemorrhages. Kidneys showed interstitial nephritis, intratubular haemorrhages and glomerular haemorrhages.

Interstitial nephritis in which infiltration of mononuclear cells and polymorphonuclear cells along with coagulative necrosis of renal tubules were also seen in most of cases (Fig.2). In few cases, fatty changes were seen. Kidneys also showed intratubular haemorrhages and glomerular atrophy. Kidneys revealed glomerular coagulative necrosis.

Kidneys exhibited protein cast in tubules, glomerular coagulative necrosis with disruption of bowman capsule and tubular necrosis with degenerated renal tubules. Cloudy swelling was also seen in few cases. Kidneys exhibited vacuolar degeneration, desquamation of epithelial cells in lumen of tubules with mononuclear and polymorphonuclear cells infiltrations in interstitium of renal tubules.

Discussion

Grossly, kidneys were swollen, congested, soft and pulpy in consistency.

Microscopically, glomerular necrosis was found in 32 cases (48.48%). A higher incidence was observed by Hassanein *et al*. (2017) [2] who reported 69.23%. Renal tubular necrosis was found in 49 cases (74.24%). A higher incidence was observed by Hassanein *et al*. (2017) [2] who reported 100%. A lower incidence was reported by Rahnemoon *et al*. (2019) [13] who reported 65% acute tubular necrosis. Fatty change was observed in 2 cases (3.03%). Congestion and oedema was found in 39 cases (59.09%). Haemorrhages were found in 45 cases (68.18%). Inflammatory cells infiltrations were observed in 63 cases (94.45%). Vacuolar degeneration was observed in 6 cases (9.09%), protein cast in 1 case (1.51%), and cloudy swelling in 7 cases (10.60%).

Histopathologically, kidneys showed congestion, necrosis, inter tubular and interstitial haemorrhage. These findings are in conformation with the findings of Khan *et al.* (2008) [7], Hines (2013) [4], Sasikala *et al*. (2016) [15] and Hassanein *et al.* (2017) [2].

Some of section showed fatty changes. This observation is in conformity with the finding of Wise (1957) [20]. Some of sections revealed oedema. This finding is similar to the finding of Khan *et al.* (2008) [7].

Gross findings are in close approximation to the findings recorded by Wise (1957) [20], Uzal and Songer (2008) [16] and Hines (2013) [4]. Some sections showed interstitial nephritis. This is in conformation with the finding of Sasikala *et al*. (2016) [15].

Few cases revealed cloudy swelling. Similar lesion is described by Mekathoti (2018) [10]. Few cases exhibited protein cast in renal tubules. This observation is in close approximation to the finding recorded by Luciano *et al.* (2010) [9].

Some sections showed vacuolar degeneration. This finding is similar to the finding of Salvarani *et al*. (2019) [14]. Few cases revealed desquamation of epithelial cells in tubules. This finding is in close approximation to the findings recorded by Khan *et al.* (2008) [7].

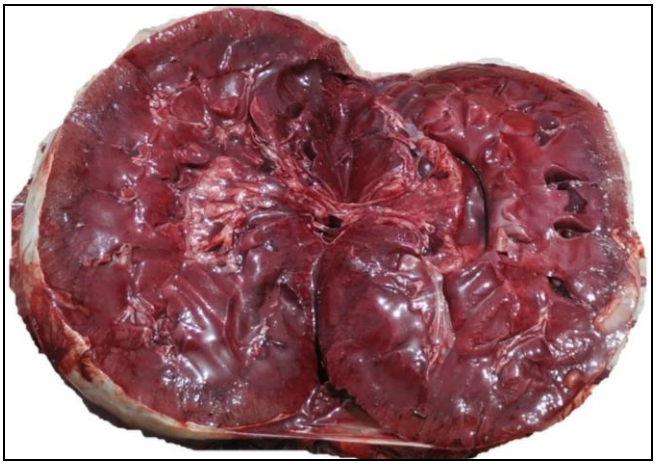

Fig 1: Gross photo showing cut section of pulpy kidney in sheep

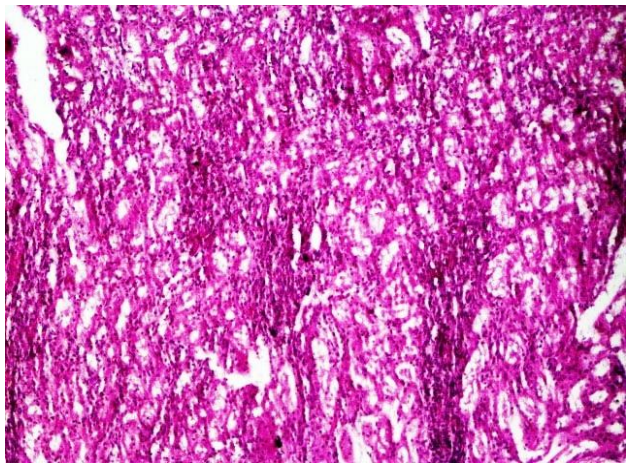

Fig 2: Microphotograph of kidney showing interstitial nephritis (mononuclear and polymorphonuclear cells infiltrations in interstitium of tubules) along with coagulative necrosis of renal tubules. H& E,100X.

Conclusion

It is a severe disease of small ruminants that develops as an outbreak, and responsible for causing huge economic losses

worldwide. Therefore, it can only be prevent by following proper vaccination and balanced diet.

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