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Histopathological study of *Clostridium perfringens* type D infection in sheep

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Abstract
Clostridium perfringens type D infection is an acute toxaemic fatal disease of sheep of all ages and is recognized worldwide. The present study was conducted to describe the gross and histopathological changes observed in *Clostridium perfringens* type D infection in sheep. In the present investigation, a detailed necropsy was performed on 362 sheep irrespective of age, sex and breeds. Out of these, 66 sheep found positive on the basis of gross and histopathological examination of carcasses. Grossly, kidneys were swollen, darkly congested, and appeared soft and pulpy in consistency. Liver was haemorrhagic. Heart showed haemorrhages, and congestion. Microscopically, kidneys showed degenerative, necrotic and iniflammatory changes. Focal coagulative necrosis of hepatocytes was seen in liver. Massive infiltration of mononuclear cells and polymorphonuclear cells was seen around the central vein. Liver revealed fatty changes, edema, and congestion in sinusoidal space. Sections of heart showed haemorrhages between the myocardial fibres. Some of sections showed congestion and mononuclear and polymorphonuclear cells infiltration in between the cardiac muscle fibres with separation of myocardial fibres.

Keywords: *Clostridium perfringens* type D, histopathology, necropsy, sheep

Introduction
Clostridium perfringens type D causes enterotoxemia, in lambs and sheep of all ages, is rapidly fatal disease. The peracute clinical form is characterized by sudden death without premonitory sign. Acute form is very rapid. Sheep usually develop a more chronic form. *Clostridium perfringens* type D is usually reside in the soil of farmlands where it is able to persist for long periods by the formation of spores. Green feed which is low in fibre and high in moisture content is the principal predisposing factor. Fiber and exercise stimulate bowel activity and when these factors are lacking or not fully operative, it slows down the movements of the bowel which make predispose the animal for enterotoxaemia. Similar effects show by the consumption of dry peas, fallen grain or a ration of concentrates. (Toop, 1957) [11]. After getting favorable conditions, it multiplies in the small intestine and secrets powerful toxin where these are absorb through bloodstream and circulates throughout the various organs resulting death of sheep within the space of a few hours. The diagnosis of enterotoxaemia is achieved by pathological findings observed in various organs. The objective of this study was to describe the gross and pathological findings observed in *Clostridium perfringens* type D infection.

Materials and Methods
Collection of samples for histopathology
The affected tissue samples of kidneys, liver and heart 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.
During this study necropsy was performed on 362 sheep irrespective of age, sex and breeds. After postmortem examination and recording of gross findings in tissues viz., kidney, liver and heart were collected in 10% neutral buffered 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. The tissue sections of 4-6 micron thickness were cut and stained with hematoxylin and eosin staining method as a routine.

Results and Discussions

During this study post mortem examination was done on 362 sheep irrespective of age, sex and breeds. On the basis of gross and histopathological examination 66 sheep found positive for this disease.

Kidney-

Grossly, kidneys were swollen, dark and congested that appeared soft, pulpy and jam like 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%. 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 (95.45%), vacuolar degeneration in 6 cases (9.09%), protein cast in 1 case (1.51%), and cloudy swelling observed in 7 cases (10.60%).

Kidneys showed congestion, oedema, inter tubular and interstitial haemorrhages (Fig.1). Some of section showed coagulation necrosis. These findings were in conformation with the findings of Sasikala *et al*, (2016) [9] and Hassanein *et al*. (2017) [2].

Some of sections showed fatty changes. This observation was in conformity with the finding of Wise (1957) [13]. Few cases revealed cloudy swelling. Similar lesion was described by Mekathoti (2018) [7]. Few cases exhibited protein cast in renal tubules. This observation was in close approximation to the finding recorded by Luciano *et al*. (2010) [6].

Some sections showed interstitial nephritis. This was in conformation with the finding of Sasikala *et al.* (2016) [9]. Some sections showed vacuolar degeneration. This finding was similar to the finding of Salvarani *et al.* (2019) [9]. Few cases revealed desquamation of epithelial cells in tubules. This finding was in close approximation to the findings recorded by Khan *et al.* (2008) [4].

Gross findings were in close approximation to the findings recorded by Wise (1957) [13], Uzal and Songer (2008) [12], and Hines (2013) [3].

Liver

Grossly, liver was hemorrhagic.

Microscopically, necrosis was found in 13 cases (19.69%). Higher incidence was observed by Hassanein *et al*. (2017) [2] who reported 100%. Congestion and oedema was found in 54 cases (81.81%). Inflammatory cells infiltrations were observed in 58 cases (87.87%) and fatty changes were found in 2 cases (3.03%).

Congestion, oedema, (Fig.2) infiltration of mononuclear and polymorpho nuclear cells, and necrosis was seen. Similar microscopic lesions were described by Sasikala *et al*. (2016) [9], Hassanein *et al.*, (2017) [2], and Salvarani *et al.* (2019) [9].

Some of cases showed fatty changes. This finding was also described by Wise (1957) [13], and Singh *et al*. (2017) [10].

Gross findings were in close approximation to the findings recorded by Singh *et al*. (2017) [10].

Heart

Grossly, heart showed haemorrhages, and congestion.

Microscopically, congestion was observed in 48 cases (72.72%). Haemorrhages were recorded in 46 cases (69.69%). Inflammatory cells infiltrations were observed in 56 cases (84.84 %).

Congestion, and inflammatory cells infiltration were noticed. Some of section showed haemorrhages between the myocardial fibres. Microscopic findings were in conformity with Kumar *et al*. (2019) [4], and Mekathoti (2018) [7].

Gross findings were similar to those described by Gokce *et al*. (2007) [1], Uzal and Songer (2008) [12], and Sasikala *et al.* (2016) [9].

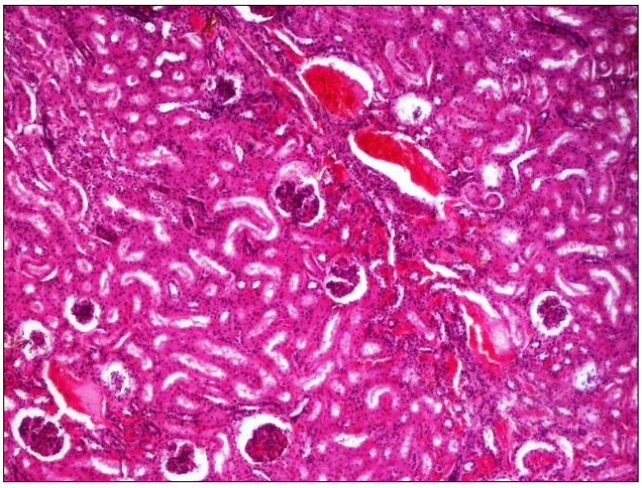

Fig 1: Microphotograph of kidney showing massive intratubular haemorrhages and intertubular haemorrhages along with glomerular haemorrhage and edema. H&E, 100X.

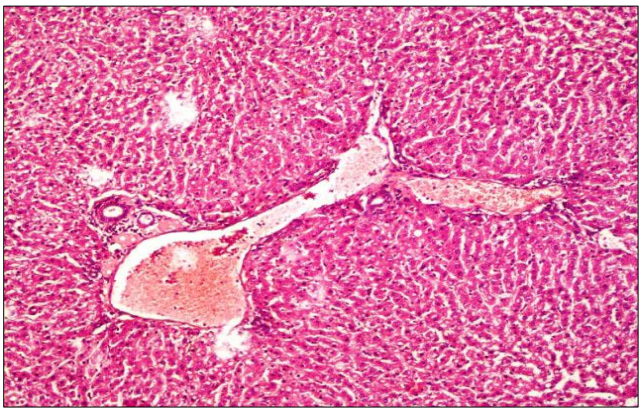

Fig 2: Microphotograph of liver showing congestion in portal vein and periportal edema. H&E, 100 X.

Conclusion

It is concluded that enterotoxemia caused by Clostridium perfringens type D is highly fatal in livestock. It is diagnosed by gross and pathological examination in various organs and proper and accurate diagnosis can prevent economic losses in sheep industries.

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