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Gross and histopathological alterations in the liver of sheep affected with diarrhea: A comprehensive study

[1]Rahul Kumar Sharma, [2]Gunjan, [3]Goverdhan Singh and [4]Ritesh Limbat

[1-4] Department of Veterinary Pathology, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

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Corresponding Author: Rahul Kumar Sharma

Abstract
The objective of this study was to investigate the pathology of liver of 22 sheep affected by diarrhoea, focusing on samples obtained from dead sheep carcasses with a history of diarrhoea. In the liver, various histopathological changes were observed, including congestion, hemorrhages, chronic venous congestion, and degenerative changes such as fatty changes, cloudy swelling, hydropic degeneration, and amyloid infiltration. Additionally, major histopathological changes in the liver encompassed coagulative necrosis, acute hepatitis, and chronic hepatitis.

Keywords: Diarrhoea, liver, pathology, coagulative necrosis

1. Introduction
The sheep husbandry system in India has predominantly adhered to traditional practices with sheep grazing on pastures facing significant susceptibility to various parasitic diseases. Among these, gastrointestinal parasitism stands out as a major health concern, leading to a spectrum of consequences ranging from diminished productivity to increased mortality. Diarrhoea, characterized by heightened frequency, fluidity, or volume of faecal excretion, is a prevalent manifestation of these parasitic infections.
The effective growth of the livestock industry hinges largely on the prevention and control of diseases among animals. A crucial aspect of this endeavor is addressing the health challenges faced by sheep, as diseases in these animals result in substantial economic losses across the milk, meat, and wool sectors.
The current study focuses on investigating the pathological changes in the liver of sheep affected by diarrhoea. Understanding the intricacies of these changes is pivotal for developing strategies to mitigate the impact of parasitic diseases on sheep health. By unraveling the pathological aspects, we aim to contribute valuable insights that can inform the formulation of targeted measures for the prevention and control of diarrhoea and its associated repercussions in sheep populations.

2. Materials and Methods
The samples for the present study were collected from various sheep farms and veterinary clinics in the southern region of Rajasthan. Additionally, sheep carcasses submitted to the Department of Veterinary Pathology, College of Veterinary and Animal Science, Navania, Udaipur, which had a history of diarrhoea, were included in the study for post-mortem examination. The post-mortem examination of 22 sheep carcasses with a history of diarrhoea was conducted under aseptic conditions from January 2020 to December 2020. All necessary precautions were taken during the post-mortem examination.
Samples for histopathology were collected during the post-mortem examination. A systemic post-mortem examination of the 22 sheep carcasses was performed to detect the presence of any lesions in the gastrointestinal tract. The liver of the deceased animals was thoroughly examined, and the observed changes were recorded. Representative tissue pieces from organs revealing lesions were collected in 10 percent buffered formalin for histopathological examination. The fixed tissue underwent a process that involved washing in running tap water, dehydration in acetone, clearing in xylene, and embedding in paraffin wax (melting point 60º-62 °C) following the method described by Lillie in 1965 [5]. Paraffin blocks were prepared, and sections were cut at a thickness of 4-5 µm using a rotary-type semi-automatic microtome. The paraffin-embedded sections were then deparaffinized using xylene, rehydrated through descending grades of ethyl alcohol to running tap water, and stained using routine hematoxylin and eosin stain (Luna, 1968) [6]. The prepared slides were examined under a microscope.

3. Results
During the gross examination of the liver, various lesions were observed in the present study. Congestion was noted in 17 cases, and haemorrhages with congestion were observed in 5 cases, with 2 cases exhibiting pinpoint haemorrhages. One case showed an enlarged liver with imprints of ribs on the surface, and in 6 cases, white greyish necrotic foci were grossly observed. An abscess with casemated pus was noticed in 1 case.
In cases of congestion, the central vein and nearby sinusoids

were filled with red blood cells (RBCs). Chronic venous congestion was observed in some cases, with the central vein distended and round. Hepatocytes around the central vein showed degenerative changes of varying degrees in a few cases.

Haemorrhages along with congestion were observed in 5 cases, showing extravasation of RBCs in the hepatic parenchyma along with infiltration. In some cases, haemorrhages were accompanied by focal areas of necrosis, with loss of cellular details and architecture, along with severe neutrophil infiltration. Dilation of engorged blood vessels and sinusoids, severe diffuse necrobiotic changes in hepatocytes with perivascular mononuclear inflammatory cell infiltration were also observed in some cases.

Degenerative changes were noted in the liver parenchyma in 16 cases, along with hepatitis. Observed changes included fatty changes, cloudy swelling, hydropic degeneration, and amyloid infiltration. Fatty changes were characterized by severely congested sinusoids and vascuolar structures in hepatocytes, where the nucleus was pushed to the periphery, resembling signet ring-like structures. Cloudy swelling featured swollen hepatocytes with fine granulation in the cytoplasm and reduced sinusoidal space. Hydropic changes showed several small fluid-filled vacuoles in hepatocytes with granular cytoplasm. Amyloid infiltration was characterized by amyloid deposition around dilated central veins, exhibiting a homogenous pink staining and a glassy appearance. Severe deposition of amyloid around the central vein and in sinusoidal space causing pressure atrophy on nearby hepatocytes was observed in some cases. Acute hepatitis was observed in 9 cases, characterized by congestion of blood vessels and infiltration of neutrophils and lymphocytes in liver parenchyma, around the central vein, blood vessels, and bile duct. Coagulative necrosis was observed as focal necrotic foci in 5 cases. Chronic hepatitis along with fibrosis was also observed in 2 cases, characterized by connective tissue proliferation in liver parenchyma and infiltration of macrophages in liver parenchyma.

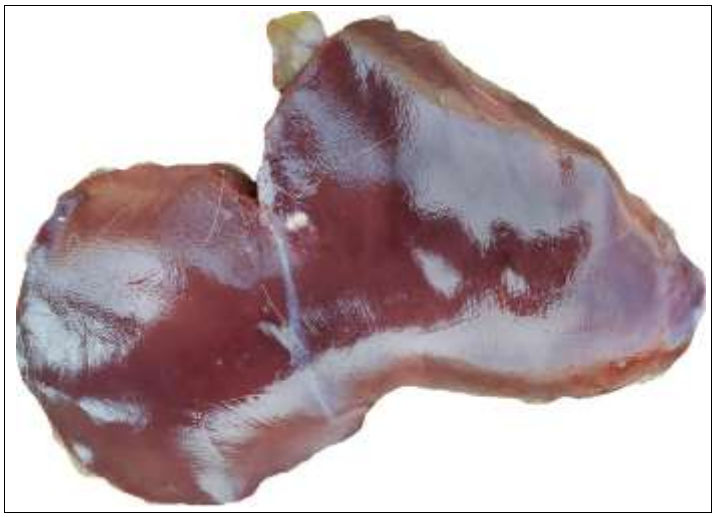

Fig 1: Gross photograph of liver showing petechial or ecchymotic haemorrhages on the surface

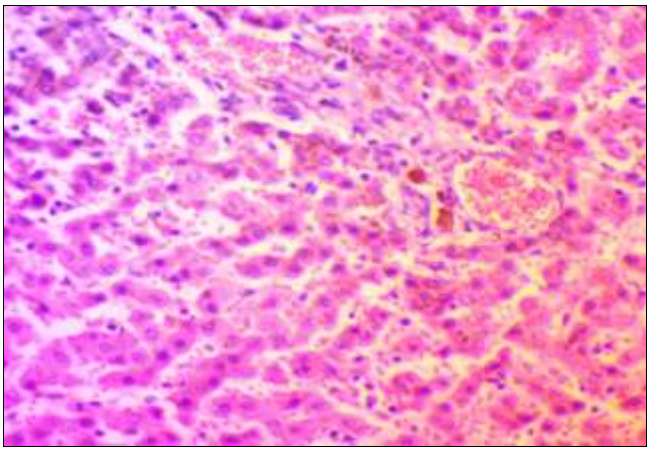

Fig 2: Microphotograph of liver showing congestion in liver with hemosiderosis, golden-brown colour, H&E-400x

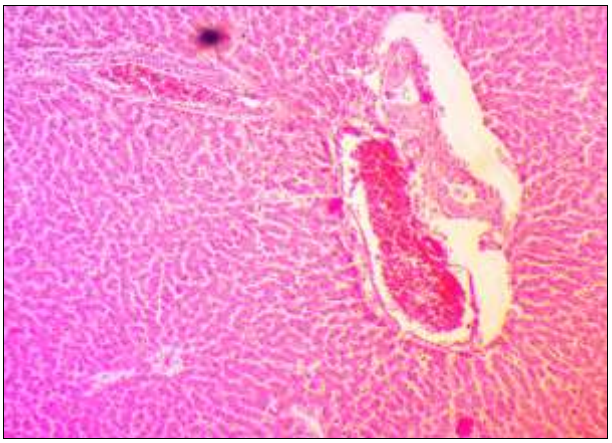

Fig 3: Microphotograph of liver showing congestion and portal vein engorged with RBC, H&E 400x

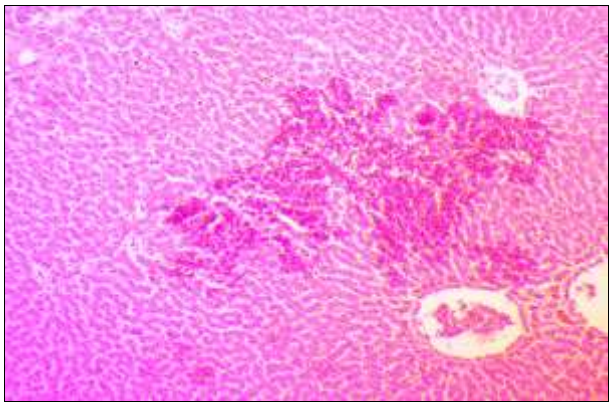

Fig 4: Microphotograph of liver showing haemorrhages extravasation of RBC in and around central vein, H&E-100x

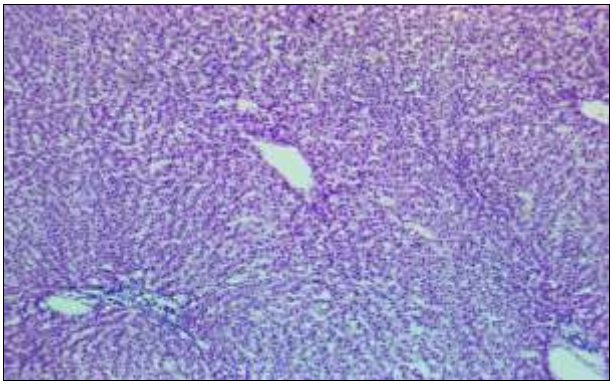

Fig 5: Microphotograph of liver showing fatty change, numerous vacuoles are seen in liver, hepatocyte nucleus pushed at periphery, looking like signet ring, H&E-100x

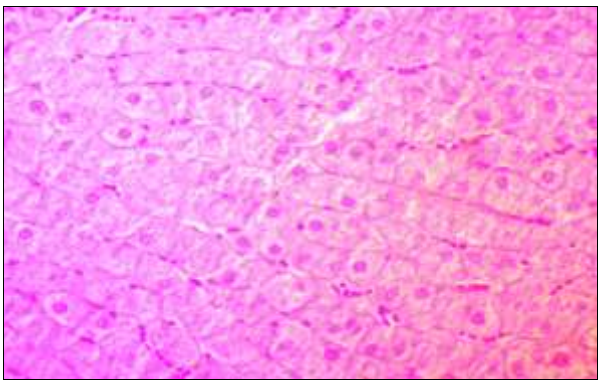

Fig 6: Microphotograph of liver showing cloudy swelling hepatocytes are swollen with fine granulation in the cytoplasm and decreased sinusoidal space, H&E -400x

Fig 7: Gross photograph of liver showing enlarged liver with imprint of ribs

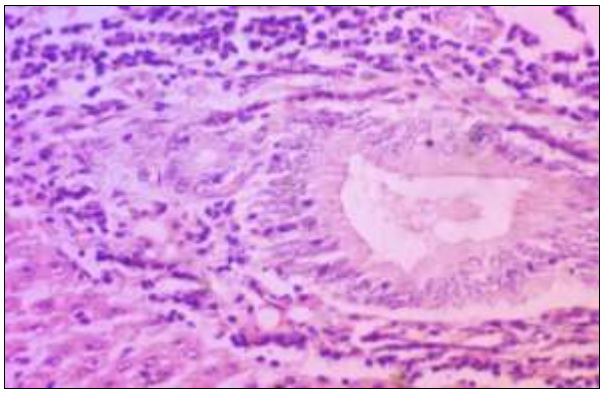

Fig 8: Microphotograph of liver showing peri-portal hepatitis and deposition of inflammatory cell at periphery of portal vein. H&E-1000x

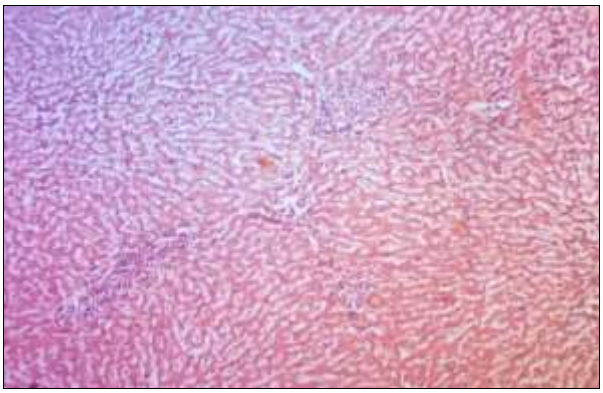

Fig 9: Microphotograph of liver showing Infiltration and necrosis H&E-100x

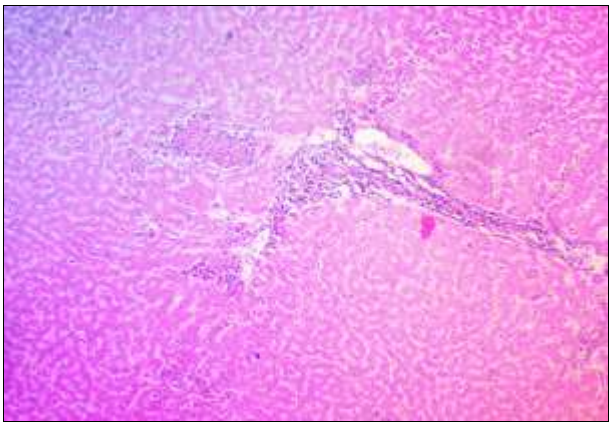

Fig 10: Microphotograph of liver showing areas of focal fibrosis H&E-100x

4. Discussions

In the present study, postmortem examinations were conducted on 22 sheep/lamb carcasses received for postmortem analysis. During the gross examination of the liver, various lesions were observed, including congestion, hemorrhages with congestion, white greyish necrotic foci, and abscess. Similar findings in the liver with diarrhoea in lambs were reported by Balachandran *et al.* (2010) [1] and Raghavendra, S.V. *et al*., (2018) [7].

Histopathological examination of the liver revealed congestion in most cases, with central veins and nearby sinusoids filled with red blood cells. Chronic venous congestion was observed in some cases, where the central

vein appeared distended and round. Hemorrhages, along with focal areas of necrosis showing loss of cellular details and architecture, as well as severe neutrophil infiltration, were noted in a few cases. Degenerative changes in the liver parenchyma, including fatty changes, cloudy swelling, hydropic degeneration, and amyloid infiltration, were also observed. Fatty changes were characterized by severe sinusoidal congestion, vacuolar structures in hepatocytes with nuclei pushed to the periphery, and signet ring-like structures throughout the field. Cloudy swelling featured swollen hepatocytes with fine granulation in the cytoplasm and reduced sinusoidal space. Similar findings were reported in earlier studies by Causape *et al*. (2002) [2], Kumar *et al*. (2015) [4], and Chaudhary *et al*. (2015) [3].

Hydropic changes in the liver included the observation of several small fluid-filled vacuoles in hepatocytes with granular cytoplasm. Amyloid infiltration showed amyloid deposited around dilated central veins with a homogenous pink staining and a glassy appearance. Acute hepatitis was characterized by congestion of blood vessels, infiltration of neutrophils and lymphocytes in liver parenchyma, around the central vein, blood vessels, and bile duct. Coagulative necrosis was observed as focal necrotic foci, in agreement with earlier reports by Tafti and Mansourian (2008) [8].

Chronic hepatitis along with fibrosis was also observed, characterized by connective tissue proliferation in liver parenchyma and infiltration of macrophages. These findings align with earlier reports by Kumar *et al*. (2015) [4] and Raghavendra *et al*. (2018) [7].

Conclusion

The present study was undertaken 22 samples were taken from dead sheep carcasses died with the history of diarrhoea. In the liver, various histopathological changes were observed, including congestion, hemorrhages, chronic venous congestion, and degenerative changes such as fatty changes, cloudy swelling, hydropic degeneration, and amyloid infiltration. Additionally, major histopathological changes in the liver encompassed coagulative necrosis, acute hepatitis, and chronic hepatitis.

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