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Aetio-Pathological studies of digestive and respiratory affections in lambs

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Abstract

Aetio-pathological studies of digestive and respiratory affections were undertaken in lambs received for post mortem examination during a period of seven months (September, 2015 to March, 2016). Maximum mortality was seen in age group of 1 to 3 months. Sex-wise mortality was more in males as compared to female lambs. System-wise causes of death/mortality were highest due to involvement of digestive system alone followed by combined involvement of both the digestive and respiratory systems and respiratory system alone. Gastritis, enteritis, pneumo-enteritis, hepatitis and pneumonia were the main conditions encountered in lambs. Microbiological study of different samples collected from carcasses of lambs revealed that maximum bacterial species isolated was *E. coli* followed by *Proteus* spp., *Klebsiella* spp. and *Salmonella* spp. Maximum numbers of bacterial strains were isolated from intestine followed by lungs, tracheal swab and heart blood. The results of *in-vitro* drug sensitivity to different bacterial species isolated from carcasses of lambs revealed that most of bacterial strains were found sensitive to gentamycin and resistant to tetracycline. Examination of faecal samples of diarrhoeic/diseased and dead lambs revealed presence of mixed infection of *Strongyle* spp. along with *Strongyloid* spp. and *Eimeria* spp.

Keywords: *Eimeria* spp., *in-vitro* drug sensitivity, lambs and mortality

1. Introduction

The sheep are economically important livestock species, contributes greatly to the Indian economy, especially in arid, semi-arid and mountainous regions. This species play an important role in the livelihood of small and marginal farmers and economically weaker sections. Rearing of young ones is extremely delicate and therefore requires a lot of care especially regarding prevention of diseases. Diseases of young ones results in heavy financial losses, which may occur, not only in the form of mortality but also because of decreased productivity from recovered lambs of both the sexes. A number of infectious, nutritional and metabolic disorders lead to heavy mortality in lambs, inflicting great economic losses to poor farmers. Among these causes gastrointestinal tract and respiratory disorders play a vital role, causing high mortality and morbidity affecting the profits in sheep production programmes. The early morbidity and mortality represents an important source of economic loss to the farmer. Bacteria responsible for mortality in lamb are *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens* and *Staphylococcus aureus*. Some of the important diseases of lambs are colibacillosis, enterotoxaemia, botulism, vibrio dysentery, salmonellosis, enterotoxaemia, pneumonia, bloat and diarrhoea. Gastrointestinal parasitism is also considered as a major challenge for the health and the welfare of lambs. Parasitism, especially by helminthic parasites, impairs health by causing inappetance, diarrhoea, anaemia and, in severe cases, death. The nematode parasite, *Haemonchus contortus*, is singly the most important nematode pathogen of small ruminants (sheep and goats) throughout the world. Correctly determining the cause of death permits one to apply effective measures to prevent further loss. Keeping in view the above facts, aetio-pathological studies on digestive and respiratory affections in lambs were undertaken.

2. Materials and Methods

Postmortem examination was carried out on 16 lamb carcasses aged below six months that were presented to the Department of Veterinary Pathology, LUVAS, Hisar and at Central Sheep Breeding Farm, Hisar during September, 2015 to March, 2016. Following parameters were studied:

2.1. Postmortem Examination and Pathological Study

A detailed postmortem examination was conducted immediately on arrival of the carcass. Representative and appropriate tissue pieces from intestine, stomach part, liver, lung, trachea, heart and mesenteric lymph nodes were collected in 10% buffered formalin and processed for histopathological studies (Luna, 1968).

2.2. Bacteriological Study

At necropsy, material for bacteriological studies was collected aseptically in sterile containers. Isolation of organisms was attempted from the heart blood, lung, trachea, and intestinal contents. The collected samples were put in nutrient broth or buffered peptone water and incubated at 37°C for 24 h. The next day 1 ml of culture from buffered peptone water was transferred to selenite broth and incubated at 37°C for 24 to 48 h, while those collected in nutrient broth were inoculated on nutrient agar (NA) and MacConkey's Lactose agar (MLA) plates and incubated at 37°C for 24 to 48 h. From MLA plate, lactose fermenting colonies were taken, inoculated on Eosin methylene blue (EMB) agar and incubated at 37°C for 24 h and the isolates were stored in maintenance medium. From NA plates, golden orange colored, round, opaque and luxuriant colonies developed were selected and inoculated on Baird Parker agar and incubated at 37°C for 24 to 48 h. From Baird Parker agar, the black colored colonies were selected and stored in maintenance medium. The culture from selenite broth was streaked on Brilliant Green agar (BGA) and incubated at 37°C for 24 h. The plates were observed for colonies after incubation. From BGA plate, pinkish white colonies were taken and inoculated on Xylose-lysine deoxycholate agar (XLD) agar and incubated at 37°C for 24 h and the isolates were stored in maintenance medium. Identification of all isolates was done following the procedure of Cruickshank and McCartney (1965) [5]. All bacterial isolates were stained by Gram's staining and examined for their morphological characteristics. Biochemical tests IMViC (Indole, methyl red, Voges-Proskauer and citrate utilization test), sugar fermentation tests, nitrate reduction test, urease tests, H₂S production test on triple sugar iron medium, and catalase test were also performed.

2.3. In-vitro Drug Sensitivity Testing

Different isolated bacterial strains were subjected to *in-vitro* drug sensitivity testing using antimicrobials by the disc diffusion method as suggested by Bauer *et al.* (1966) [3]. With the help of a platinum loop, small amount of growth from at least three isolated colonies of the organisms were transferred into a tube of trypticase soya broth and incubated for two to five hours at 37°C so as to obtain a turbidity, equivalent to that obtained by adding 0.5 ml of 0.048 M BaCl₂ (1.175 % BaCl₂, 2H₂O) to 99.5 ml of 0.36M NH₂SO₄ (1 % v/v). The broth culture was then evenly spread by smearing over the surface of Mueller Hinton agar plates. Different antibiotic discs of standard concentrations were then used. The plates were then incubated at 37°C for 18 to 24 h and observed for sensitivity by measuring the zones of inhibition. Results were noted as sensitive (S) and resistant (R) on the basis of the table provided by the manufacturer (Himedia) for zone size interpretation.

2.4. Parasitological Studies from Faecal Samples

A total of 26 faecal samples were collected from both dead (16) and diarrheic (10) lambs from the organized farm of the

University; Central Sheep Breeding Farm, Hisar and postmortem hall of the Department of Veterinary Pathology, LUVAS, Hisar. Examination of these faecal samples was performed as per method of Soulsby (1982) [26] by floatation and sedimentation methods.

3. Result and Discussion

As per the information provided, most of the animals had died suddenly with no clinical signs and symptoms, while others were being treated for diarrhoea, dehydration and weakness. Table 1 depicts the mortality pattern according to age, sex, system-wise causes of death/mortality, bacterial species isolated from different samples and faecal examination of diarrhoeic/diseased and dead lambs. The proposed study starting from September, 2015 to March, 2016 in lambs revealed that age-wise mortality was maximum in age group of 1 to 3 months followed by 3 to 6 months and no mortality was seen in age group of upto 1 month. Reddy and Choudhuri (1999) [22] also reported maximum mortality during second month of life in lambs. In contrary to this, Khan *et al.* (2006) [14] reported that mortality was at peak with in the first week of life. Sex-wise mortality was more in male as compared to female lambs. Similar findings were revealed by Mandal (2007) [17] and Tibbo *et al.* (2010) [27] whereas Reddy and Choudhuri (1999) [22] did not find any significant difference in mortality, on the basis of sex. Mortality was highest due to involvement of digestive system alone followed by combined involvement of both the digestive and respiratory systems and respiratory system alone. Gastritis, enteritis, pneumo-enteritis, hepatitis and pneumonia were the main conditions encountered in lambs. More or less similar observations were reported by Reddy and Choudhuri (1999) [22]; Ramirez-Briebesca *et al.* (2001) and Mandal (2007) [17] in lambs.

Gross pathological changes observed during postmortem examination of lambs shown in Table 2. Grossly intestine revealed congestion (Fig.1) and presence of catarrhal exudate. Mesenteric lymph nodes were found swollen and enlarged. Abomasum divulged presence of petechial haemorrhages (Fig.2) in mucosa. Liver showed congestion, necrotic foci, firmness, induration and cyst. Gall bladder was distended. Lungs revealed congestion (Fig.3), consolidation and exudation of pus from cut surface. Daniel *et al.* (2006) [6] also revealed consolidation of lung tissue in lambs. Heart showed haemorrhages on epicardium. Kidneys were darkly congested having multiple necrotic foci in focal areas. Spleen revealed petechial to ecchymotic haemorrhages. Urinary bladder was found distended (Fig.1, 4). Apart from above, hydrothorax and Sero-sanguinous fluid in abdomen (Fig.4) were also observed. More or less similar findings were reported by Abou-Zaid *et al.* (2000) [1] in lambs.

Histopathologically, intestine exhibited enteritis characterised by infiltration of leucocytes and degeneration of mucosal glands (Fig.5). Desquamation of mucosal epithelium and goblet cell hyperplasia, necrosis of villi, congestion in mucos and submucosa were also observed. Abomasum disclosed abomasitis characterized by necrosis of glands in mucosa, leucocytic cell infiltration and congestion. These microscopic observations were similar to findings of Richards *et al.* (1993) [23] and Gough and Mc Even (2000) [10]. Liver showed necrotic hepatitis characterised by mild perivascular cell infiltration and coagulative necrosis, severe congestion in blood vessels and dilated sinusoids (Fig.6). Few cases were showing hepatitis characterised by vacuolative changes in hepatocytes. In some cases, focal areas of necrotic hepatitis characterised

by centerilobular coagulative necrosis along with mononuclear cells infiltration was observed. Similar lesions in liver have also been reported by Patel *et al.* (2015) [20]. Lungs divulged suppurative pneumonia characterised by presence of sero-purulent exudate in alveoli, infiltration of leucocytes and emphysema (Fig.7). Congestion and haemorrhages in parenchyma was also seen. Serous pneumonia characterised by presence of haemorrhages, serous fluid in alveoli and multifocal area of leucocytic cells infiltration was seen in some of the cases. Other changes noticed were hemosiderosis and red hepatization. Trachea revealed mild tracheitis characterised by presence of haemorrhages and mild leucocytic cells infiltration. Almost similar lesions in lung have also been reported by Hazirolu *et al.* (1994) [11]; Oruc (2006) and Patel *et al.* (2015) [20]. Spleen showed haemorrhages and depletion of lymphocytes in white pulp along (Fig.8) with haemosiderosis in red pulp area. Necrosis of splenic tissue in medullary region along with presence of haemorrhages in parenchyma and congestion in blood vessels was also noticed. Mesenteric lymph nodes revealed depletion of lymphocytes in cortical area and congestion in medullary area. Similar lesions in spleen and mesenteric lymph node have been reported by Panisup (1974) and Som and Bhattacharya (1987) [25] in lambs. Heart showed presence of sarcocysts, congestion of blood vessels, fragmentation and degeneration of muscle fibres. Apart from these, mild fatty and hydropic changes were also observed. Kidney disclosed interstitial nephritis characterised by degeneration of tubules along with presence of leucocytic cells infiltration in the interstitium and congestion as well as in glomeruli capillaries. Degeneration of tubules, infiltration of leucocytes in inter-tubular area and fatty changes were observed in kidney in few cases of lambs. Similar lesions have been reported by Hussain *et al.* (1986) [13] and Deepti *et al.* (1999) [7]. Microbiological study of different samples collected from

carcasses of lambs revealed that maximum bacterial species isolated was *E. coli* followed by *Proteus* spp., *Klebsiella* spp. and *Salmonella* spp. Maximum numbers of bacterial strains were isolated from intestine followed by lungs, tracheal swab and heart blood. Similar observations were reported Abou-Zaid *et al.* (2000) [1]; Genc (2002); Wani *et al.* (2003) [28] and Ahmed *et al.* (2010) [2] regarding the isolation of different bacterial species from lambs. One *E. coli* strain belongs to serotype O118. It is appropriate to mention here that serotype O118 has been found to be zoonotic in nature as it produces Human Uremic Syndrome (HUS), haemorrhagic colitis in human being as reported by Schlundt (2002) [24]. *In vitro* drug sensitivity revealed that *E. coli* was found highly sensitive to gentamycin, ciprofloxacin; *Salmonella* spp. to gentamycin, streptomycin; *Klebsiella* spp. to cefotaxime, amoxicillin; *Proteus* spp. to cefotaxime, amikacin. *E. coli* was found highly resistant to tetracycline, *Salmonella* spp. to polymyxin B, tetracycline, amoxyclav, ampicillin; *Klebsiella* spp. to amoxyclav. *Proteus* spp. to polymyxin B, tetracycline and amoxyclav. Almost similar observations with respect to antimicrobial sensitivity resistance patterns have also been reported previously by Blanco *et al.* (1996) [4]; Abou-Zaid *et al.* (2000) [1]; Orden *et al.* (2000); Wani *et al.* (2004) [29] and Mahmoud *et al.* (2013). Examination of faecal samples of diarrhoeic/diseased lambs revealed presence of mixed infection of *Strongyle* spp. along with *Strongyloid* spp. and *Eimeria* spp., *Strongyle* spp. along with only *Strongyloid* spp. There was also presence of *Strongyle* spp. and *Eimeria* spp. alone. Examination of faecal samples from dead lambs revealed mixed infection of *Strongyle* spp. along with *Strongyloid* spp., *Strongyle* spp. along with *Strongyloid* spp. and *Eimeria* spp., *Strongyloid* spp. along with *Eimeria* spp. and *Eimeria* spp. alone. These findings are in accordance with those of Ghanem *et al.* (2005) [9] they noticed oocysts of *Eimeria* spp. in faeces of lambs.

Table 1: Mortality pattern according to age, sex, system-wise causes of mortality, bacterial species isolated from different samples and faecal examination of diarrhoeic/diseased and dead lambs

Age and sex-wise mortality in lambs						
Age groups	Males (Number of cases)	Percentage of age-wise mortality in males	Females (Number of cases)	Percentage of age-wise mortality in females	Total	Percentage of total age-wise mortality
Up to 1 month	0	00.00	0	00.00	00	00.00
1-3 months	10	66.66	1	100.00	11	68.75
3-6 months	5	33.34	0	00.00	05	31.25
Total number of sex-wise mortality	15(93.75%)	-	1(6.25%)	-	16 (100.00%)	
System-wise causes of death/mortality in lambs						
System-wise causes of death/mortality	Digestive system alone	Respiratory system alone	Combination of both digestive and respiratory systems	Others systems/ causes	Putrefied carcass	Total number of cases
Total number of system-wise mortality	10(62.50%)	1 (6.25%)	5 (31.25%)	-	-	16 (100.00%)
Bacterial species isolated from different samples collected from lambs						
Bacterial species isolated	Intestine	Lungs	Tracheal swab	Heart blood	Total number of different bacterial species isolated	Percentage of different bacterial species isolated
<i>E. coli</i>	16	2	0	3	21	55.26
<i>Klebsiella</i> spp.	5	0	0	0	5	13.16
<i>Salmonella</i> spp.	1	0	3	0	4	10.52
<i>Proteus</i> spp.	3	5	0	0	8	21.06
Total number of bacterial species isolated from lambs	25(65.78%)	7(18.42%)	3(7.90%)	3(7.90%)	38 (100.00%)	
Faecal examination in diarrhoeic/diseased lambs						

<i>Strongyle</i> spp.	<i>Eimeria</i> spp.	Mixed infection	Negative sample	Total number of cases
2	2	4 (<i>Strongyle</i> spp. + <i>Strongyloid</i> spp. + <i>Eimeria</i> spp.) 2 (<i>Strongyle</i> spp. + <i>Strongyloid</i> spp.)	0	10
Faecal examination in dead lambs				
<i>Strongyle</i> spp.	<i>Eimeria</i> spp.	Mixed infection	Negative sample	Total number of cases
0	1	4 (<i>Strongyle</i> spp. + <i>Strongyloid</i> spp.) 3 (<i>Strongyle</i> spp. + <i>Strongyloid</i> spp. + <i>Eimeria</i> spp.) 1 (<i>Strongyloid</i> spp. + <i>Eimeria</i> spp.)	7	16

Table 2: Gross pathological changes observed during postmortem examination of lambs

Gross Changes	Intestine	Liver	Mesenteric lymph nodes	Abomasum	Lungs	Heart	Kidneys	Spleen	Urinary bladder	Gall bladder
Congestion	11	9	-	-	6	-	6	-	-	-
Haemorrhages	-	-	-	2	-	2	-	4	-	-
Necrotic foci	-	2	-	-	-	-	2	-	-	-
Firmness and Induration	-	1	-	-	-	-	-	-	-	-
Consolidation	-	-	-	-	2	-	-	-	-	-
Cysts	-	1	-	-	-	-	-	-	-	-
Enlargement	-	-	5	-	-	-	-	-	-	-
Exudate	3 (Catarrhal)	-	-	-	1 (Pus)	-	-	-	-	-
Distension	-	-	-	-	-	-	-	-	3	1

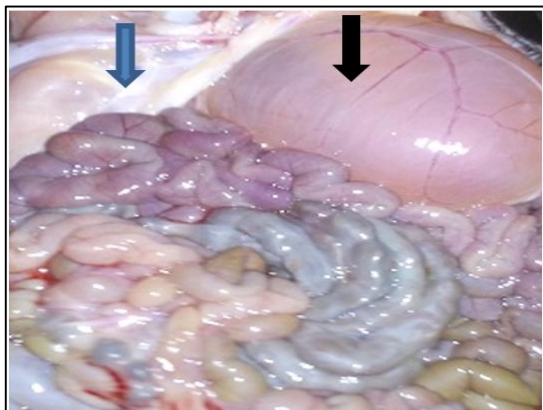


Fig. 1: Congested intestine (blue arrow) and distension of urinary bladder (black arrow) (Lamb, *E. coli*, *Klebsiella* spp., *Eimeria* spp. and *Fasciola* infection)



Fig. 3: Congestion in lungs (Lamb, *E. coli*, *Klebsiella* spp., *Strongyloid* spp. and *Eimeria* spp. infection)

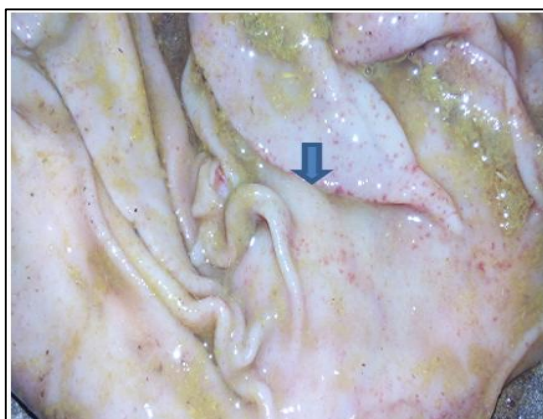


Fig.2: Petechial haemorrhages in abomasal mucosa (Lamb, *E. coli*, *Strongyle* spp. and *Strongyloid* spp. infection)

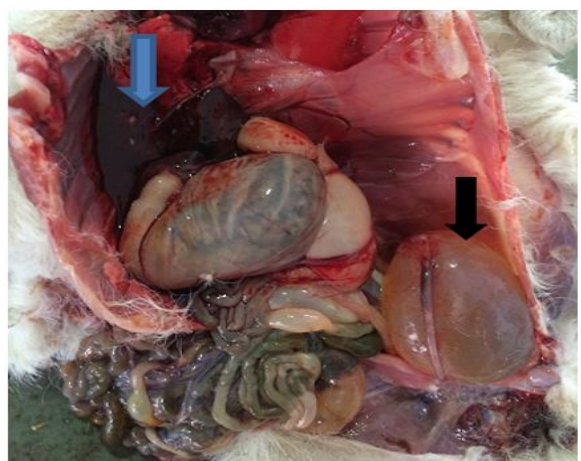


Fig. 4: Sero-sanguinous fluid (blue arrow) in abdomen along with distended urinary bladder (black arrow) (Lamb, *E. coli* and *Proteus* spp. infection)

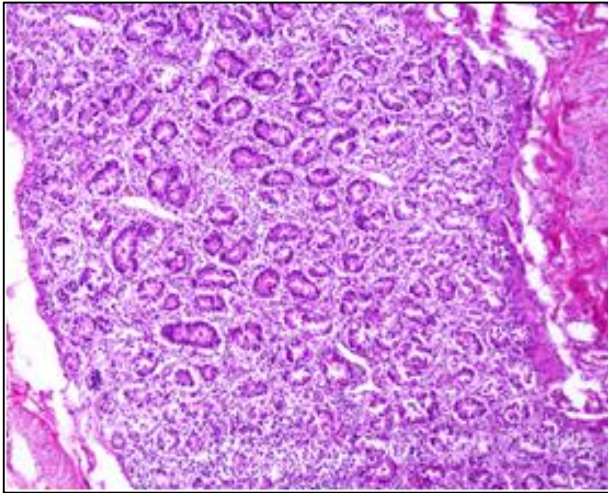


Fig. 6: Intestine: Enteritis characterised by infiltration of leucocytes in mucosa and degeneration of mucosal glands (Lamb, *Salmonella* spp., *Strongyle* spp., *Strongyloid* spp. and *Eimeria* spp. infection) H & E x 100

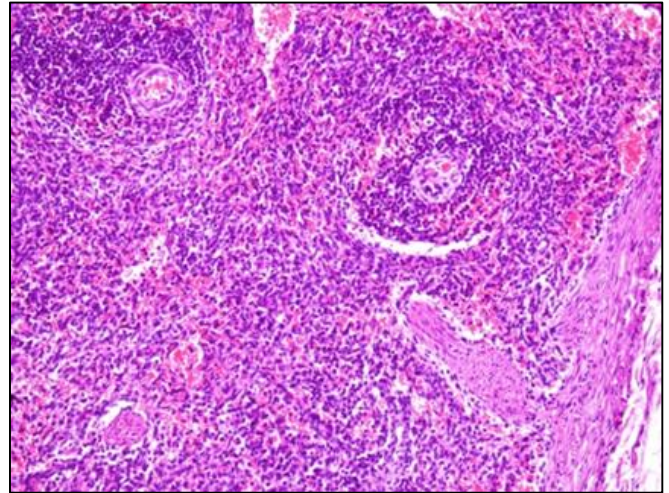


Fig. 8: Spleen: Haemorrhages and depletion of lymphocytes in white pulp (Lamb, *Salmonella* spp., *Strongyle* spp., *Strongyloid* spp. and *Eimeria* spp. infection) H & E x 100

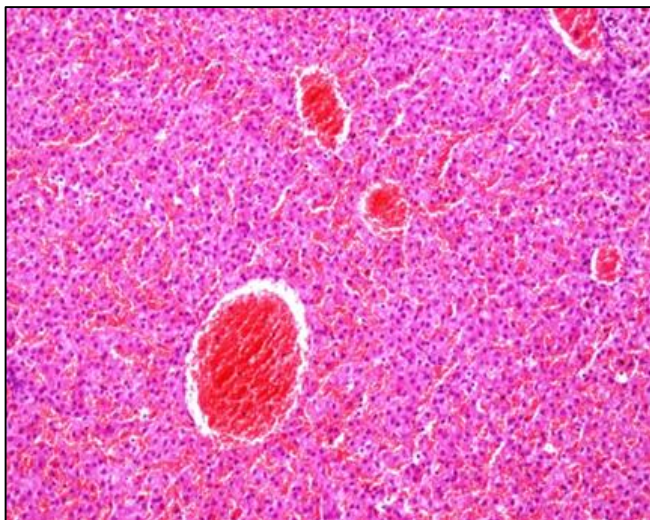


Fig. 7: Liver: Hepatitis characterised by severe congestion in blood vessels and in dilated sinusoids (Lamb, *E. coli* and *Klebsiella* spp. infection) H & E x 200

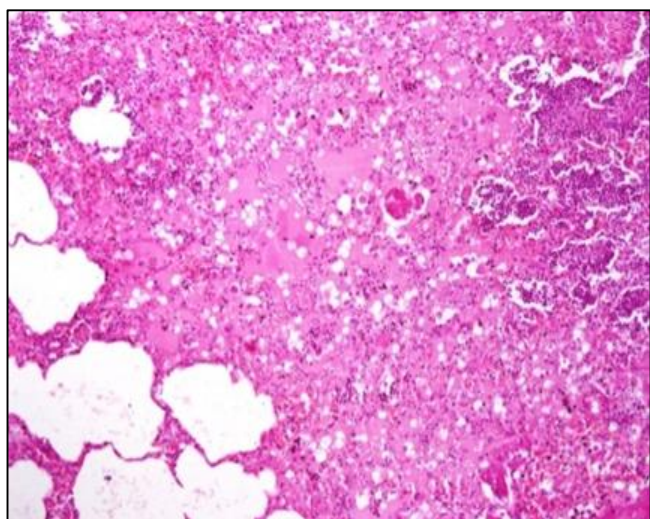


Fig. 7: Lungs: Suppurative pneumonia characterised by presence of sero-purulent exudate in alveoli, infiltration of leucocytes and emphysema (Lambs, *Salmonella* spp., *Strongyle* spp., *Strongyloid* spp. and *Eimeria* spp. infection) H & E x 100

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Conflict Of Interest

Authors declare that there is no conflict of interest regarding the present research work.

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