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Incidence and mortality due to digestive diseases in rabbits

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Abstract
In the present study, tissue samples from rabbit carcasses collected from organized farms located in and around Hyderabad apart from the animals necropsied in the Department of Veterinary Pathology, PVNRTVU, College of Veterinary Science, Rajendranagar, Hyderabad. A total of 150 rabbit carcasses of either sex and of different age groups died during January to July 2018 were subjected to detailed postmortem examination for pathological and bacteriological study. System wise causes of mortality was summerized in which mortality due to involvement of digestive system is 34.99%. In digestive system, gastric ulcer was observed in one (2.85%) case, enteritis was noticed in 8 (22.85%) cases. Hepato pathology was found to be very common and was recorded in 26 (74.3%) out of 35 carcasses among 150 carcasses examined. Various hepato-pathological conditions such as degenerative changes (17.14%) like fatty change (8.57%) and necrosis (8.57%), acute hepatitis (24.28%), chronic hepatitis (17.71%), cholangio hepatitis (11.53%) and hepatic tumor (7.70%) were recorded. Combined lesions of both respiratory and digestive systems are seen in 17 carcasses (11.33%) in which pneumo enteritis (41.17%) and pneumo hepatitis (58.82%) were noticed. A total of 45 samples from lungs and intestines were examined for bacterial isolation and the pathogens isolated were *Staphylococcus* spp. (33.33%), *E. coli* (24.43%), *Pasturella* spp. (8.9%) and *Salmonella* (11.11%), *Klebsiella* (6.7%) *pseudomonas* (2.22%) respectively. ABST revealed that most of the bacterial strains were sensitive to gentamycin and enrofloxacin.

Keywords: mortality, hepatic diseases, enteritis, rabbits, bacteriological studies and ABST

Introduction
India as a developing country, is thriving for balanced nutritional food security. Rabbit meat stands a good chance to serve as a prospective alternative meat source owing to its obvious qualities. Now a days rabbit farming is one among the fastest growing livestock industry. However, an intensive system of rabbit production may lead to physiological and environmental stresses. Ultimately the stress factors results in entry of pathogens and led to different disease conditions which affects growth performance, feed efficiency and health status of rabbits [26]. The literature on the mortality pattern and various pathological conditions in rabbits is spare in India particularly Telangana state. Moreover the prevalence of digestive and respiratory diseases are very common comparative to other systemic infections in rabbits [5, 28, 31]. Therefore, the present study was proposed to study the gross and histopathological lesions in diseases of digestive system along with isolation and identification of organisms.

Materials and Methods
Source
The materials for the present study consisted of tissue samples from rabbit carcasses collected from organised farms located in and around Hyderabad apart from the animals necropsied in the Department of Veterinary Pathology, PVNRTVU, College of Veterinary Science, Rajendranagar, Hyderabad. A total of 150 rabbit carcasses of either sex and of different age groups were necropsied, and respective sample were collected for histopathological and bacterialogical study.

Collection of samples
Postmortem examination and pathological studies
At necropsy, a detailed examination was carried out on rabbit carcasses for the presence of

gross lesions if any. Tissue slices from representative portions of lungs, stomach, intestines, liver that showed definite gross lesions were collected and fixed in 10% neutral buffered formalin (NBF) for histopathological examination.

Bacteriological examination

For bacteriological studies tissue samples and swabs were collected aseptically in sterile container. Samples were collected randomly from suitable organisms.

Laboratory procedures

Histopathology

The pieces of lung, stomach, liver and intestine showing gross lesions were collected in 10% NBF for fixation and processed for histopathological studies, by routine paraffin embedding technique [14].

Bacterial isolation

The swabs were collected in sterile test tubes and inoculated in nutrient broth/Brain

Heart Infusion (BHI) broth and incubated at 370C for 24 hours. Then the broth culture material was streaked on nutrient agar and BHI agar plates prepared in microbiology laboratory as per manufacturer's instructions. A provisional identification of bacterial growth was done based on the colony morphology and Gram's staining. Isolation and characterization of bacteria was done as mentioned in Bergey's Manual of Determanative Bacteriology [9]. The selective media for different bacterial species *viz*. Mac Conkey agar for gram negative bacteria, Eosin Methylene blue (EMB) agar for E.coli, Mannitol salt agar for Staphylococcus spp and sheep blood agar media for Pasteurella spp were used.

Antibiogram

Preparation of 0.5 McFarland standards

Mc Farland Standard 0.5 was prepared by adding 0.5 mL of 1% barium chloride dehydrate (BaCl2, 2H2O) to 9.5mL of 1% sulphuric acid (H2SO4) by constant stirring. Mc Farland Standard was used as reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize the microbial antibiotic sensitivity testing. McFarland Standard No.0.5 gives a standard inoculums size of approximately 1-2 x 108 C.F.U/mL. The test was performed by using Antibiotic test discs manufactured by Hi-mediaLaboratories limited, Mumbai and Oxoid, UK with principle of disc diffusion method of Kirby and Baeur [3].

Materials

Mueller-Hinton agar was used to study the antibiotic sensitivity pattern of the isolates.

Results and Discussion

In the present study, digestive system affections were noticed in 35 cases (23.33%) out of 150 rabbits. Pathomorphological observation revealed liver affections [26] followed by enteritis [8] and ulcers in stomach [1]. Digestive disorders are the predominant cause of mortality in commercial rabbits [24, 42]. The losses in the rabbits after weaning were mostly due to lack of fibre in diet and infection like coccidia or bacterial proliferation [24]. Earlier studies [20, 22] concluded enteritis was the second major cause of mortality in kits. Enteropathogenic E. coli (EPEC) is one of the most important causes of losses in broiler rabbits in large scale farms [21] and it may be due to lack of balance of intestinal flora during post weaning [25]. Gastric ulcer was seen in 1 rabbit (2.85%), usually these ulcers results from trauma, infection, circulatory disturbances and uraemia etc., but in present study, the ulcer was associated with trichobezoars. Gastric trichobezoars to be responsible for (43%) death in New Zealand white rabbits due abnormal grooming behavior of individually caged rabbits [43]. On the other hand, agents that reduce mucosal prostaglandin synthesis cause mucosal injury, especially in the stomach, and may result in gastric ulcer [11]. Grossly, ulcers were clearly demarcated with raised borders and punched out appearance with depressed necrotic center which was also observed [15]. Microscopically, the epithelial cells were desquamated along with severe congestion in submucosa and leukocytic infiltraton. These ulcers were characterized by acute inflammatory exudates and fibrinoid necrosis. These results were in accordance with earlier studies [8, 29]. In the present study enteritis was recorded in 8 rabbits and the incidence was less compared to the earlier reports [30, 38]. The cause of enteritis could be attributed to dietary changes, stress factors or infectious agents. The lesions were characterised grossly by congested and thickened intestinal mucosa and microscopically necrosis of villi and congestion in submucosa (Fig. 1). Earlier workers [7, 17, 10] reported higher incidence of coccidial enteritis which was not recorded in the present study. This might be due to scrupulous maintenance of rabbits in cages.

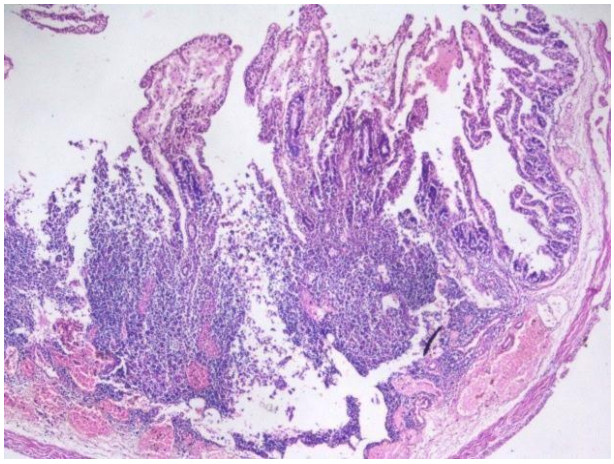

Fig 1: Photomicrograph of rabbit intestines showing necrotic villi and leucocyticinfiltration in mucosa. H&E X 400

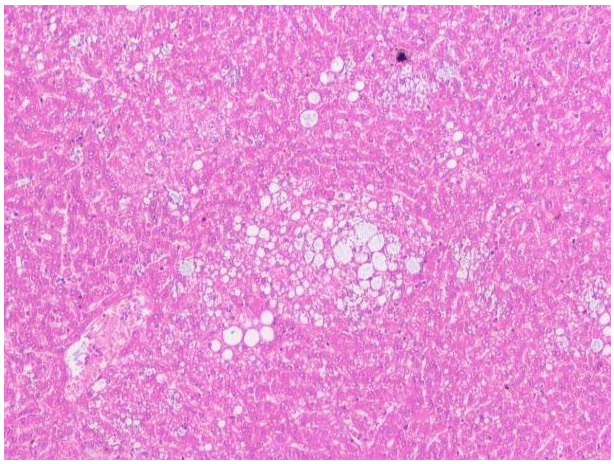

Fig 2: Photomicrograph of liver showing numerous small clear vaccuoles representing fatty change in liver. H&E X 100

In the present study, liver affections (74.3%) were quite common finding in rabbits examined at post mortem examination. More or less similar occurrence (75-80%) of liver affections has been reported [27, 28, 38]. Fatty changes were encountered in 3 (8.57%) cases out of 26. Grossly, liver appeared pale with rounded borders. Microscopic observation revealed presence of small clear vacuoles in the cytoplasm and nucleus was pushed to periphery (Fig. 2). Hepatic lipidosis is usually trigged by anoxia which can be caused by many factors including pain, fear, change in diet and other health problems [19]. The gross and microscopic findings were similar to recent reports [13]. Centrilobular necrosis with changes around the central vein was encountered in (5.71%) of livers examined. This lesion might be due to bacterial toxins conveyed to the liver by blood flow from the intestines. Grossly, liver showed varying sized necrotic foci. Microscopically, congestion and necrosis around the central vein was observed (Fig. 3). Acute hepatitis was observed in 24.23% cases and grossly liver showed congestion. Microscopically, congestion of central vein, dilatation of sinusoids, perivascular lymphoid aggregates was observed (Fig. 4).

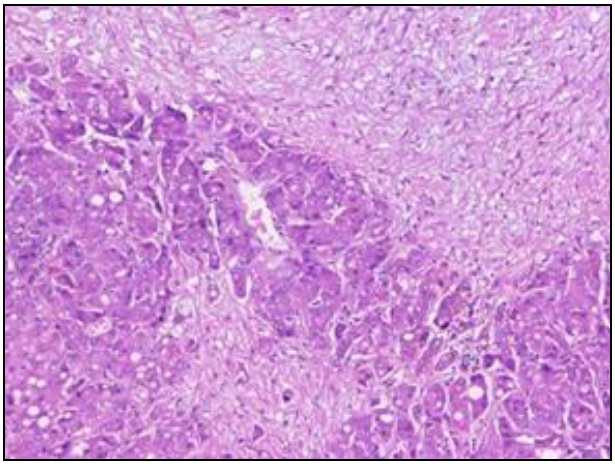

Fig 3: Photomicrograph of rabbit liver section showing congestion and necrosis around the central vein. H&E X100.

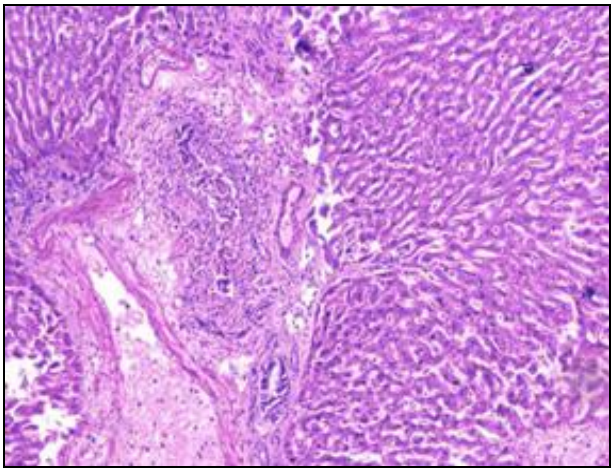

Fig 4: Photomicrograph rabbit liver showing sinusoidal dilatation, leucocytic infiltration in portal triad, bile duct epithelial cells showing degenarative changes. H&E X100.

Chronic hepatitis (cirrhosis) was noticed in 17.71% of livers examined. Grossly, liver was shrunken and nodular and microscopically, proliferation of fibrous tissue along with lymphocytic infiltration in portal area and hepatic parenchyma along with bile duct hyperplasia and proliferation of new bile ducts in portal area in liver was noticed (Fig. 5). Biliary cirrhosis in rabbits is a toxic effect caused by some environmental factors [41]. Cirrhosis with bile duct proliferation was also reported in rabbits fed with aflatoxins contaminated feed [12, 18, 33, 38]. Cholangio hepatitis was observed in 3 (11.53%) cases. Grossly, liver was enlarged and surface was smooth and greenish in color. Microscopically, bile ducts were dilated with degenerative changes of the epithelium and surrounded by connective tissue with inflammatory cells. The hepatic parenchyma adjacent to this showed degenerative changes (Fig. 6).

Hepatic tumor was found in 2 (7.70%) out of 26 carcasses showing liver affections. Grossly, liver was enlarged and had focal, round nodules of the tumor with 6x4x2.5 cm and 33 g weight. Microscopically, liver parenchyma showed abnormal growth of cells with loss of normal architecture arrangement. The proliferating neoplastic cells showed mitotic figures and hyperchromatic nuclei. Inaddition, multiple bile ducts with retention of bile in the lumen and lymphocytic infiltration was observed in the portal triad (Fig. 7, 8). The tumor was diagnosed as Hepato cellular carcinoma.

In the present study, some miscellaneous conditions (3.33%) like trichobezoars in stomach [1] and abdominal hernia [2] were observed. In trichobezoars condition, hair balls were found in stomach surrounded by mucus and microscopically, congestion of gastric mucosa along with some hemorrhages were observed. The condition of abdominal hernia was found in 2 rabbits in which abdominal organs were protruded out through a small artificial opening at caudal portion of peritoneum.

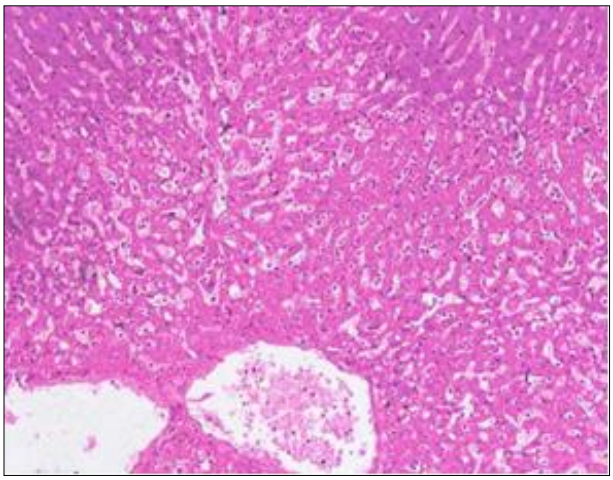

Fig 5: Photomicrograph showing proliferation of fibrous tissue along with lymphocytic infiltration in portal area and hepatic parenchyma. H&E X100

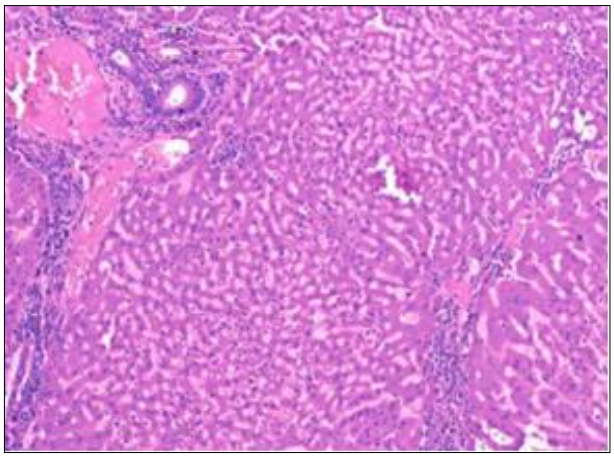

Fig 6: Photomicrograph showing dilatation of bile duct along with epithelial degeneration. H&E X100

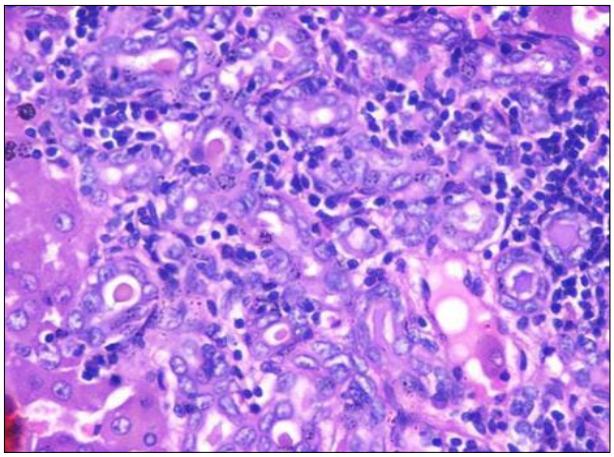

Fig 7: Photomicrograph of liver showing multiple bile ducts and heavyleucocytic infiltration in the portal triad. H&E X 400.

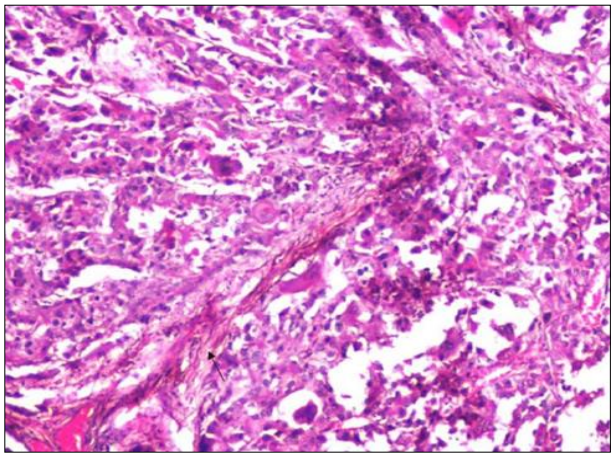

Fig 8: Photomicrograph of rabbit liver showing abnormal growth of cells and hyperchromatic nuclei. H&E X 100.

Adhesions between visceral mass and the hernial sac causing accumulation of ingesta in intestines were observed. Microscopically, congestion of intestines along with heamorrhages were noticed. Combined lesions in respiratory and digestive system was encountered in 17 cases (11.33%), grossly pneumonia and enteritis was recorded in 7 cases and pneumonia along with hepatitis was noticed in 10 rabbit carcasses. Microscopically, pneumonia was characterised by haemorrhages, edema and thickened alveolar septa along with severe leucocytic infiltration in bronchiolar & alveolar lumen. Intestines exhibited enteritis was characterized by congestion in mucosa, goblet cell hyperplasia and leucocytic infiltration in the submucosa. Hepatocytes showed coagulative necrosis along with leucocytic infiltration. These lesions were in accordance with earlier studies [20].

Bacteriological studies

Microbiological samples collected from lung samples revealed some pathogens like Staphylococcus spp. 15 (33.33%) (Fig. 9), E.coli 11 (24.43%) (Fig. 10), Pasturella spp. 4 (8.9%). Similarly common bacteria isolated from intestines were Salmonella spp. 5 (11.11%), Klebsiella spp. 3 (6.7%) (Fig. 11) and Pseudomonas spp. 1 (2.22%) respectively. Some earlier studies [2, 4, 6, 7, 16, 23, 34-39] reported isolation of the similar bacterial species from lungs and intestines of rabbit carcasses.

The result of *in vitro* drug sensitivity to different bacterial species isolated from lungs and intestines revealed that Staphylococcus spp. was sensitive to gentamicin, enrofloxacin, ceftrioxone and resistant to ampicilline and methicilline. E.coli sensitive to gentamycin, ceftrioxine, ampicilline and resistant to cloxacilline, celfoperazon. Salmonella spp. sensitive to ceftrioxine, celfoperazone, gentamycin and resistant to ampicilline, enrofloxacine (Fig. 12), Klebsiella spp. sensitive to ampicilline and enroflaxacine resistant to ceftrioxine, celfoperazone and methicilline (Fig. 13).

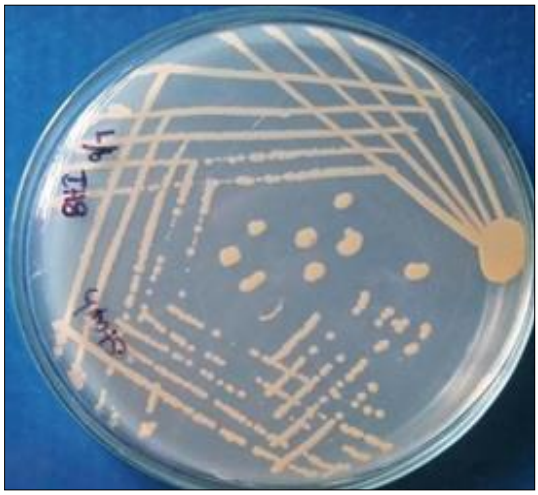

Fig 9: Photograph showing small golden yellow opaque colonies of Staphylococcuson BHI agar

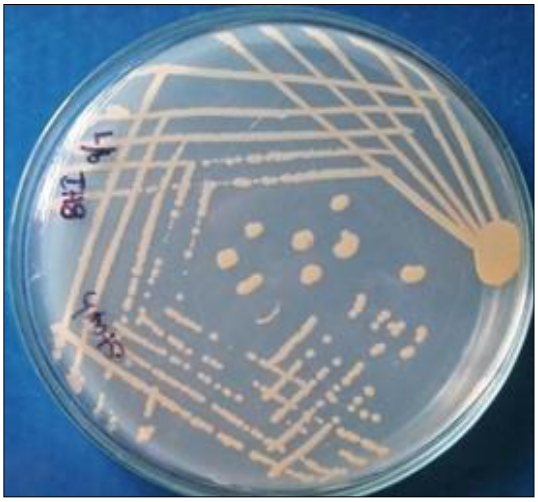

Fig 10: Photograph showing pink colour colonies by E. coli on MacConkey agar

Pseudomonas spp. sensitive to gentamycin and enrofloxacin where as resistant to ampicilline, cloxacilline, celfoperazone and ceftrioxine. Almost similar results with respect to antimicrobial susceptibility resistance patterns have been reported previously [1, 32, 40].

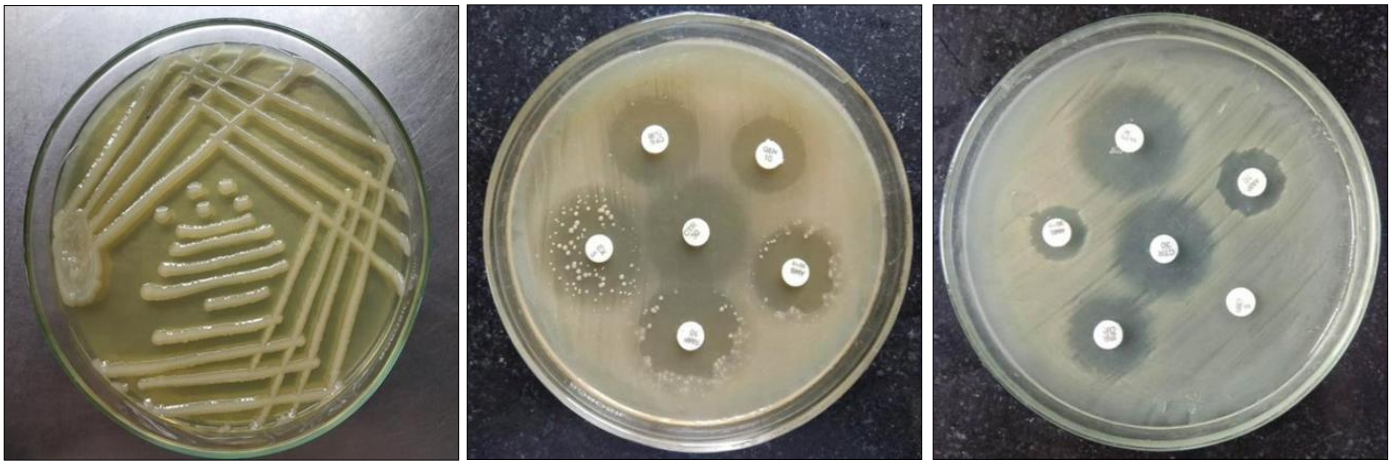

Fig 11: Photograph showing Mucoid colonies of *klebsiella* on Nutrient agar

Conclusion

In conclusion, mortality due to involvement of digestive system in rabbits is 34.99% in which the gastric ulcer was observed in one (2.85%) case, enteritis was noticed in 8 (22.85%) cases. Hepato pathology was found to be very common and was recorded in 26 (74.3%) out of 35 carcasses among 150 carcasses examined. Various hepato-pathological conditions such as degenerative changes (17.14%) like fatty change (8.57%) and necrosis (8.57%), acute hepatitis (24.28%), chronic hepatitis (17.71%), cholangio hepatitis (11.53%) and hepatic tumor (7.70%) were recorded. Combined lesions of both respiratory and digestive systems are seen in 17 carcasses (11.33%) in which pneumo enteritis (41.17%) and pneumo hepatitis (58.82%) were noticed. The bacteriolgical examination revealed major pathogens affecting the lungs were Staphylococcus spp. (33.33%), E.coli (24.43%) and Pasturella spp. (8.9%) whereas Salmonella (11.11%) was the major bacterial pathogen causing digestive system infections in rabbits along with Klebsiella (6.7%) and pseudomonas as minor pathogens in the present study for which gentamycin, ceftriaxone and enrofloxacin were showed antibiotic activity effectively.

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