

STUDY ON INCIDENCE, GROSS AND HISTOPATHOLOGICAL CHANGES ON COMMONLY OCCURRING PATHOLOGICAL CONDITIONS IN RABBITS (*ORYCTOLAGUS CUNICULUS*) OF HIMACHAL PRADESH, INDIA

RAKESH KUMAR*, RUPALI MASAND, ABHISHEK KUMAR, R.K. ASRANI and V.K. GUPTA
Department of Veterinary Pathology, DGCN COVAS CSKHP KV, Palampur-176062, India

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ABSTRACT

The present study was undertaken to document the incidence of spontaneously occurring pathological conditions in rabbits of Kangra valley in north-western himalayan region, India. In this study, the pathological conditions were recorded in 44 rabbits (male=18, female=26) presented for necropsy examination. The series of conditions recorded included affections of digestive system including hepatobiliary system (n=13, 29.5%), respiratory system (n=3, 6.8%), urinary system (n=1, 2.3%), cardiovascular system (n=1, 2.3%), pathological conditions involving combination of multiple systems (n=20, 45.4%) and miscellaneous conditions including cold stress and ear canker (n=6, 13.6%). The incidence rate of pathological conditions was highest in females (n=20, 59%) as compared to male rabbits (n=18, 40.9%). The maximum incidence of pathological conditions was reported during summer (n=16, 36.5%) followed by winter (n=14, 31.9%), monsoon (n=10, 22.8%) and spring (n=4, 9.1%) seasons. The pathological alterations were predominantly evident in digestive system (catarrhal enteritis, intestinal coccidiosis, ulcerative gastritis, liver cirrhosis etc.) followed by the affections of respiratory system (suppurative, fibrinopurulent bronchopneumonia, pulmonary emphysema, pulmonary congestion and oedema etc.), kidneys (chronic interstitial nephritis) and heart (fibrinopurulent pericarditis). The pathological conditions reported during post-mortem examination were scored and evaluated on the basis of gross and histopathological findings.

Keywords: Incidence, Himachal Pradesh, Pathological conditions, Rabbit

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Domestic rabbit (*Oryctolagus cuniculus*) besides a companion animal also reflects its emergent enterprise value as far as meat and fur production is concerned. Rabbits also signify their role in many immunological research studies like polyclonal antibodies production and often used as an experimental model in studies related to hypertension, aspiration pneumonia, tuberculosis and ageing (Cooper *et al.*, 2017). Occurrence of bacterial infections causing bronchopneumonia in rabbits is often influenced by agro-climatic factors, altitude, rain fall etc. (Sharma and Gupta, 2015). The morbidity and mortality in rabbits is often associated with multi-systemic disorder and routinely lead to potential economic losses to farmers or research laboratories (Elamin, 2013). The young rabbits are more prone to develop bacterial dysbiosis and acid production thereby causing catarrhal enteritis (Haligur *et al.*, 2009). The excessive use of antibiotics in rabbits can be a common cause for the proliferation of *Clostridium spiroforme* organisms leading to enterotoxaemia due to excessive toxins production (Hawkins and Bishop, 2012). The conditions like suppurative bronchopneumonia, heterotopic bones formation and granulomatous pneumonia are the conditions mainly depict an age related issue in the rabbits (Cooper *et al.*, 2017). Mortality in rabbits is also found to be associated with lung worms leading to respiratory distress (Sharma *et al.*, 2017). Hepatitis in rabbits can be an outcome of ascending

infection from intestine through portal vein to the liver and many etiologies like *Clostridium piliformis* and *Eimeria stiedae* are often associated to cause necrotic hepatitis (Meredith and Rayment, 2000).

The main focus of this study is to document the pathological conditions in rabbits recorded during routine necropsy investigations.

MATERIALS AND METHODS

Study area and sampling: The material for present study was collected from different rabbit farms in and around Kangra district (32° 0669 N 76° 3637' E) of Himachal Pradesh. A total of 44 rabbits (18 male and 26 females) were necropsied during a period from May 2018 to November, 2019 in the Department of Veterinary Pathology DGCN COVAS, CSKHPKV, Palampur.

Gross pathological study: The animals were thoroughly examined for pathological lesions. The gross lesions observed during necropsy examination were properly recorded and photographed. The lesions were palpated for the conformation of any cystic structure/abscess/tumor etc. For histopathological investigation 4-5 mm thick representative tissue sections containing affected and normal portions were collected in 10% neutral buffered formalin solution (NBF).

Histopathological study: Neutral buffered formalin fixed tissues were kept for overnight washing. The tissue sections

*Corresponding author: rkvvetpath@gmail.com

were dehydrated with ascending grades of alcohol, cleared in benzene and were embedded in paraffin. Sections of 0.5 μm thickness were cut and stained for histopathological investigation under light microscope (Luna, 1968). Stained tissue sections were mounted with DPX and examined for any pathological lesions observed during the microscopic evaluation and photomicro-graphed.

RESULTS AND DISCUSSION

The system showing highest prevalence rate in this study was gastro-intestinal and hepato-biliary system (29.5%) followed by respiratory (6.8%), urinary (2.3%) and cardiovascular systems (2.3%). The systems depicting pathological alterations in association with other systems showed a prevalence rate of 59.1% (Table 1). In present study death rate was high in female rabbits ($n=26$, 59.1%) in comparison to male rabbits ($n=18$, 40.9%). Female rabbits ($n=26$, 59.1%) exhibited higher mortality pattern as compared to male rabbits ($n=18$, 40.9%). The mortality rate in rabbits below 1 year of age was highest in male ($n=13$, 72.2%) than in female rabbits ($n=7$, 26.9%), whereas the animals between 1-5 years of age showed reverse pattern of mortality (male=22.2% and female=38.5%). The incidence of pathological conditions in the age group more than 5 years revealed the maximum pathological associations in females as compared with male rabbits (male=5.6%, female=34.6%) (Table 2). The highest prevalence of pathological conditions was reported during summers ($n = 16$, 36.5%) followed by winters ($n = 14$, 31.9%), monsoons ($n = 10$, 22.8%) and springs ($n = 4$, 9.1%) seasons. The digestive system disorders were reported with maximum frequency during summers followed by winter and monsoon seasons, whereas respiratory system affections were more common during winters followed by summers. The urinary system and cardiovascular systems related pathological conditions were recorded during summers and winters seasons, respectively. The involvement of multiple systems was more preferably seen during summers (6) and winters (6) followed by monsoon (5) and spring (1) seasons (Table 3). Several studies have documented the effect of variation in seasons to affect the outcome of diseases in rabbits (White *et al.*, 2001; He and Earn, 2007). Researchers have also provided the evidences of increased mortality in juvenile population affected with certain viral infections like rabbit hemorrhagic fever as compared with older rabbits. Hence, age can be a contributory factor linked to the outcome of infectious conditions in rabbits (Mutze *et al.*, 2014).

Out of a total 44 rabbits, 13 rabbits exhibited digestive system problems and 4 of the rabbits out of 13 with digestive system problems reflected catarrhal enteritis

with gelatinous mucoid contents ($n=13$, 30.8%). Similar changes were also reported earlier in rabbit farm in China (Hu *et al.*, 2018). This pathological alteration can be as a result of goblet cell hyperplasia occurred because of hyperacidity, bacterial overgrowth or micro-floral imbalance as per the studies carried by Haligur *et al.* (2009) and Rosenthal *et al.* (2011). Catarrhal to hemorrhagic fecal contents and emaciation were observed in 3 rabbits ($n=13$, 15.4%) affected with intestinal coccidiosis and showed parallel evidence with a study conducted by Oliveira *et al.* (2011). These pathological alterations caused by *Eimeria* spp. often corresponds to managerial problems including poor husbandry practices, dietary issues and many other stress factors as well (Decubellis *et al.*, 2013). In our study, out of a total 13 rabbits with digestive system problems 2 rabbits ($n=13$, 15.4%) showed moderate to severe erosive to ulcerative gastritis (Fig. 1) with hyperemic borders in association with catarrhal enteritis. This incidence of ulcerative gastritis in rabbits might be correlated with stress, higher production of pepsin and gastric acid in rabbits (Decubellis *et al.*, 2013). Histological examination of intestine elucidated eosinophilic necrotic exudate in the lumen and destruction of villous architecture in association with inflammatory cells infiltration especially heterophils and lymphocytes.

In this study, a total of 4 rabbits ($n=13$, 30.8%, 2 with cirrhosis and 2 with nodular hepatitis) were reported. The rabbits with cirrhosis showed hobnail like raised nodular lesions (Fig. 2), which on histopathological evaluation revealed marked fatty change (Fig. 3), hydropic or vacuolar degeneration (Fig. 4), multinucleated cells (Fig. 5), bile duct hyperplasia, fibrosis and areas of coagulative necrosis (Fig. 6). The development of cirrhotic changes may be attributed to mycotoxicosis (Krishna *et al.*, 1991), however the presence of mycotoxins could not be ruled out in the present study. One among these rabbits showed lemon sized embedded, raised, multifocal cystic structures on the liver surface (Fig. 7), while the other reflected the presence of hard nodular, raised lesions in different organs including serosal surface of the stomach, intestine, liver, lungs, diaphragm, kidneys and heart. Serosanguineous fluid with thick fibrinous exudate forming adhesions with peritoneal wall was also evident (Fig. 8). The histopathological examination of these nodular lesions in lungs revealed the presence of significant cellular atypia, pleomorphism, enlarged, irregular and multiple nuclei with polychromasia, and increased nuclear cytoplasmic ratio (Fig. 9).

Out of a total 3 rabbits with respiratory system problems lungs of one of the rabbit were found to be covered by creamish coloured pus like material, which on histological evaluation depicted the presence of

Table 1**System wise incidence of different pathological conditions recorded during necropsy examination of rabbits**

S.No.	System affected	Pathological conditions	Number of animals affected	Total number/ percentage of animals affected
1.	Digestive and hepato-biliary system	Catarrhal enteritis	4	13/29.5%
		Intestinal coccidiosis	3	
		Gastro-enteritis	2	
		Cirrhosis/ chronic hepatitis/fibrosis	2	
		Nodular hepatitis	2	
2.	Respiratory system	Suppurative bronchopneumonia	1	3/6.8%
		Fibrino-purulent bronchopneumonia	1	
		Pulmonary congestion and oedema	1	
3.	Urinary system	Chronic interstitial nephritis	1	1/2.3%
4.	Cardiovascular system	Fibrino-purulent pericarditis	1	1/2.3%
5.	Respiratory & digestive system	Pneumo-enteritis	8	11/25%
		Pneumo-hepatitis	2	
		Pneumo-gastritis	1	
6.	Digestive & rinary system	Gastroenteritis with nephritis	1	2/4.5%
		Entero-hepatitis with nephritis	1	
7.	Respiratory, digestive & urinary system	Pneumo-hepatitis with nephritis	2	7/15.9%
		Pneumo-entero-hepatitis with nephritis	2	
		Pneumo-enteritis with nephritis	3	
8.	Miscellaneous conditions	Cold stress	4	6/13.6%
		Ear canker	2	

*The number in parenthesis represent percentages.

degenerated cellular debris along with severe infiltration of heterophils admixed with few MNCs. One rabbit presented for necropsy in the department exhibited froth in trachea along with severe, diffuse hemorrhages of the lungs (Fig. 10). In this study, one rabbit (2.3%) exhibited the presence of pitted appearance of the kidneys indicating chronic nephritis (Fig. 11). Pitted appearance of kidneys in rabbits along with segmental granulomatous interstitial nephritis is often attributed to an infection caused by a protozoan parasite *Encephalitozoon cuniculi* (Harcourt-Brown, 2013). An adult rabbit also depicted the heart with shaggy appearance, where the pericardial sac was completely adhered with epicardial surface of the heart. On histopathological investigation the fibrinous exudate along with cellular debris and heterophilic infiltration was majorly evident (Fig. 12).

Almost in 18 (40.9 %) animals, the lungs showed involvement of other systems as well, including digestive system (intestine, stomach and liver) and kidneys. In this study formation of mucus mixed hairballs/Trichobezoars was also reported in two animals. The formation of gastric hairballs is mostly a compensatory mechanism, where the rabbits consume its own body hairs to meet the

Table 2**Sex-age wise incidence of pathological conditions in rabbits**

Sex	Age groups		
	<1 years	1-5 years	>5 years
Male (n=18, 40.9%)	13/18 (72.2%)	4/18 (22.2%)	1/18 (5.6%)
Female (n=26, 59.1%)	7/26 (26.9%)	10/26 (38.5%)	9/26 (34.6%)
Total (n=44)	20/44 (45.4%)	14/44 (31.8%)	10/44 (22.7%)

*The number in parenthesis represent percentages.

requirement of diet deficient in fiber (Mondal *et al.*, 2006). In this study a total of 7 animals (15.9%) showed the involvement of three or four systems altogether namely lungs, liver and kidneys (n=7, 28.6%); lungs, intestine, liver and kidneys (n=2, 28.6%) and lungs, intestine and kidneys (n=3, 42.9%). In two rabbits, involvement of kidneys was seen with stomach, intestine and liver. The disturbances in the microflora of intestine can lead to the production of toxins and uremic condition thereby, as recorded by a study carried by Vanholder and Glorieux (2015). Four of the young rabbits presented for postmortem examination showed severe pulmonary congestion with oedema with the history of rainfall on previous night. The animals died because of chilling stress

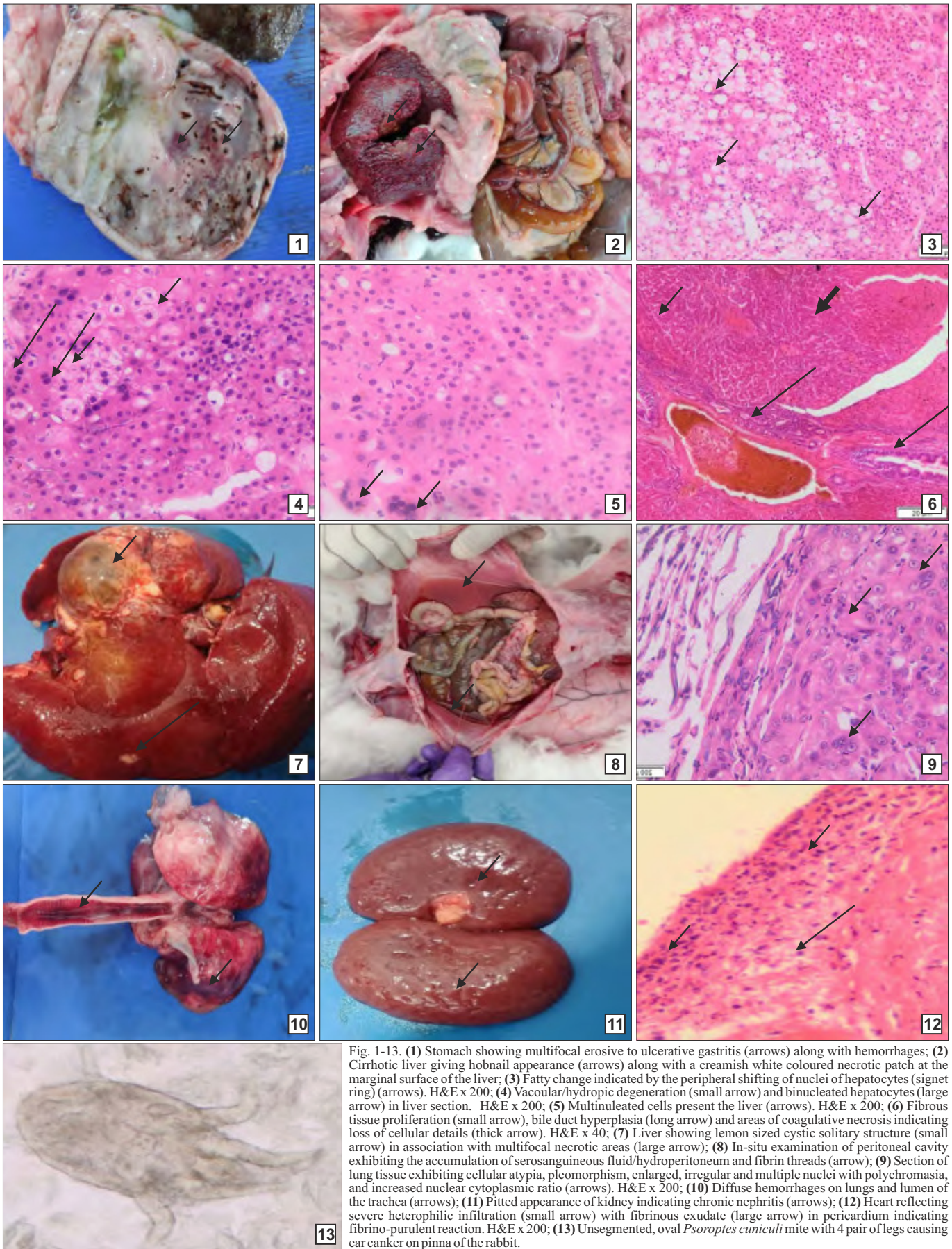


Fig. 1-13. (1) Stomach showing multifocal erosive to ulcerative gastritis (arrows) along with hemorrhages; (2) Cirrhotic liver giving hobnail appearance (arrows) along with a creamish white coloured necrotic patch at the marginal surface of the liver; (3) Fatty change indicated by the peripheral shifting of nuclei of hepatocytes (signet ring) (arrows). H&E x 200; (4) Vacuolar/hydropic degeneration (small arrow) and binucleated hepatocytes (large arrow) in liver section. H&E x 200; (5) Multinucleated cells present the liver (arrows). H&E x 200; (6) Fibrous tissue proliferation (small arrow), bile duct hyperplasia (long arrow) and areas of coagulative necrosis indicating loss of cellular details (thick arrow). H&E x 40; (7) Liver showing lemon sized cystic solitary structure (small arrow) in association with multifocal necrotic areas (large arrow); (8) In-situ examination of peritoneal cavity exhibiting the accumulation of serosanguineous fluid/hydroperitoneum and fibrin threads (arrow); (9) Section of lung tissue exhibiting cellular atypia, pleomorphism, enlarged, irregular and multiple nuclei with polychromasia, and increased nuclear cytoplasmic ratio (arrows). H&E x 200; (10) Diffuse hemorrhages on lungs and lumen of the trachea (arrows); (11) Pitted appearance of kidney indicating chronic nephritis (arrows); (12) Heart reflecting severe heterophilic infiltration (small arrow) with fibrinous exudate (large arrow) in pericardium indicating fibrino-purulent reaction. H&E x 200; (13) Unsegmented, oval *Psoroptes cuniculi* mite with 4 pair of legs causing ear canker on pinna of the rabbit.

Table 3
Season-wise incidence of pathological conditions in rabbits

Season	System affected						
	Digestive and hepato-biliary	Respiratory	Urinary	Cardiovascular	Multiple systems conditions	Miscellaneous	Total
Summer (April-June)	6	1	1	-	6	2	16 (36.4%)
Monsoon (July-Sept)	3	-	-	-	5	2	10 (22.7%)
Winter (Oct-Jan)	4	2	-	1	6	1	14 (31.8%)
Spring (Feb-March)	-	-	-	-	3	1	4 (9.1%)
Total (n=44)	13/44 (29.5%)	3/44 (6.8%)	1/44 (2.3%)	1/44 (2.3%)	20/44 (45.4%)	6/44 (13.6%)	44

*The number in parenthesis represent percentages.

leading to respiratory failure. Two of the rabbits were found with crusty ears/ear canker with the history of head shaking and nervous symptoms showed the presence of *Psoroptes cuniculi* (Fig. 13) on microscopic examination after treating the crusty material with 10% KOH as studied by Bhagat *et al.* (2017).

CONCLUSION

The goal of this study was to describe and document the incidence and pathology of commonly occurring pathological conditions in rabbits presented for necropsy examination. This study emphasizes the need of routine management of pathological conditions like cold stress in winters, trichobezoars formation in heavy concentrate diets, feed analysis for aflatoxicosis detection and regular deworming of the rabbits.

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