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Pathomorphological study on leptospirosis in slaughtered goats

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Abstract
The aim of study was to evaluate gross and microscopic lesions in leptospirosis among goats.A total of 42 goats from slaughter house were included in study and sera were collected and tested for presence of antileptospiral antibodies. All the animals were examined grossly and tissues viz. liver, lung, spleen, kidney were collected and histopathological changes in seropositive animals were noted. Warthin starry stain was also done in tissue sections to reveal presence of leptospires if any. Antileptospiral antibodies were noted in 11.90 % (5/42) goats with one or multiple serovar reactivity in microscopic agglutination test. Serovars Pyrogens, Pomona, Canicola and Hardjo were noted among goats. The histopathological changes observed in kidneys, liver, spleen and lungs were degenerative and inflammatory in nature and the kidney was found to be the most commonly affected organ in leptospirosis.

Keywords: Leptospirosis, antibodies, seropositive, histopathology

Introduction
Leptospirosis, zoonotic disease with worldwide distribution, is caused by pathogenic *Leptospira* spp. It is reported in tropical and subtropical regions of the world. Infection is noted in wide range of animals and clinically leptospirosis varies from acute to subacute or/and chronic infection especially in domestic animals. In humans or animals, the disease can result from direct contact with infected urine or tissue from infected animals or through contact with water or soil previously contaminated by urine [1].
Among goats, subclinical and/or chronic forms are common where in most infected animals show impaired fertility, abortion, stillbirth, and decreased milk production which results in heavy economic losses[2]. An acute form of leptospirosis is exhibited by pyrexia, depression, jaundice, anorexia and anemia or hemorrhagic syndromes [2].
In animals, leptospira organism can penetrate through different routes but entry via abraded skin/mucous membrane is more common. Then leptospires enter in circulation and disseminates into internal organs like kidney and liver. Organism colonizes in renal tubules which makes infected animal renal carrier. Infected animals become source of infection to other animals as they shed leptospires in the environment intermittently or continuously [3].
Confirmatory diagnosis of leptospirosis is done by isolation or molecular testing or typing of organism. Most frequently used serological test for diagnosis and to know serovars of leptospira organism is microscopic agglutination test (MAT), the "gold standard" test [4].
Leptospirosis has been reported in animals from Gujarat [5, 6, 7, 8] but scarce literature is available on pathomorphological attributes of leptospirosis in animals especially goats. Thus, the present study was aimed to determine frequency of *Leptospira* spp. in goats from slaughterhouse and to know pathological changes in infected animals.

Materials and Methods
Collection of samples and study design
Sera samples were collected from total 42 goats prior to sacrifices at Surat Municipal Slaughter House, Surat Municipality Centre, Surat, South Gujarat, India on different days. These animals were brought to slaughter house from nearby places of Surat and Navsari districts. There was no history of vaccination for leptospirosis in these animals. All sera samples were subjected to MAT to identify seropositive animals. After sacrifice, these animals were necropsied and gross lesions, if any, were critically examined/recorded in lungs, liver, kidneys and spleen in each case. The idea was to provide a holistic approach to correlate these observations. Thin slices (2-3 cm) of these morbid materials were fixed in 10 % neutral buffered formalin for histopathological examination following routine methods. The

microsections from these organs were stained by routine H & E method and also subjected to Modified Warthin Starry staining to detect the presence of leptospires in these organs.

Sera for MAT

Blood samples collected in plain vacutainers were kept in slanting position without any disturbance until serum oozed out. These vacutainers were placed in ice box and soon transported to our Veterinary Pathology Laboratory. To obtain serum the blood samples collected in plain vacutainers were centrifuged at 7800 rpm for 10 minutes. The sera were transferred into 2.0 ml sterile cryovials, aliquoted, given identification numbers and finally stored at -20ºC until further use.

MAT

All the sera were tested for antibodies against live antigens of *Leptospira* sp. serovars Pyrogenes, Australis, Bankinang, Grippotyphosa, Patoc1, Pomona, Icterohaemorrhagiae, Hebdomadis, Canicola, Hardjo, Bellum and Bataviae by MAT at Leptospira Reference Laboratory, Government Medical College, Surat using standard procedure [9]. Results were considered positive when 50% or more agglutination of leptospires at titre of 40 or more to any of the serovars was observed. A titre of 40 was used as the cut off because it was closest dilution to the usual cut off 50 used in seroepidemilogical surveys. Leptospira species included in the antigen panel are listed in Table 1.

Table 1: Panel of *Leptospira*serogroups and serovars used in MAT at Leptospirosis Reference Laboratory, GMC, Surat

Sr. No.	Sero groups	Serovars
i.	Pyrogenes	Pyrogenes
ii.	Australis	Australis
iii.	Autumnalis	Bangkinang
iv.	Grippotyphosa	Grippotyphosa
v.	Semeranga	Patoc1
vi.	Pomona	Pomona
vii.	Icterohaemorrhagiae	Icterohaemorrhagiae
viii.	Hebdomadis	Hebdomadis
ix.	Canicola	Canicola
x.	Sejroe	Hardjo
xi.	Bellum	Bellum
xii.	Bataviae	Bataviae

Histopathology

Formalin fixed tissues were processed by paraffin wax embedding method for tissue sectioning. Sections were cut at 5-6 microns and stained with haematoxyline and eosin (H & E) stain. The H & E stained slides were observed under microscope and lesions were recorded.

Special staining

Five micron paraffin sections were subjected to special staining like Modified Warthin-Starry method[10] to differentially visualize *Leptospira* organisms in the sections.

Results and Discussion

All the sera were subjected to MAT and seropositivity was found to be 11.90 % (5/42 goats) in goats with one (n=4) or more (n=1) anti-leptospiral antibodies/serovars reactivity. For pathomorphological study only MAT positive animals were taken into consideration. Details of MAT results are presented in Table 2. As such morbid materials collected from these 5 goats were subjected to gross and histopathological studies as well as special staining.

In MAT, frequently reported serovar was Pyrogens followed by each Pomona, Canicola and Hardjo. In one case, reactivity was noted against two serovars i.e. Canicola and Hardjo at different titre.

Table 2: Details of serovars reactivity at different titres in seropositive goats

Number of seropositive goats	Serovars			
	Canicola	Hardjo	Pomona	Pyrogens
1	160	640	-	-
2	-	-	1280	-
3	-	-	-	160
4	-	-	-	80
5	-	-	-	80

Presence of anti-leptospiral antibody in serum of unvaccinated animals is indicative of present or past exposure to organism. One or more serovars noted in the goats indicate circulation of these serovars in region south Gujarat. Suitability of the environment for survival of leptospires is crucial factor for maintaining the infection among animals and its transmission. Leptospirosis is endemic in south Gujarat as tropical environment, high humidity, alkaline soil, water logging and high rainfall etc. factors of this region are ideal for survival and propagation of leptospires and this may the reason for reported high frequency of leptospiral antibodies in animals.

Studies conducted in the south Gujarat, in recent past reported Pomona, Hardjo, Canicola, Bankinang in cattle [6], serovars Pomona, Canicola, Icterohaemorrhagiae in sheep[7] and serovars Pyrogenes and Tarassovi in goats [8] as predominant serovars. Similarly, in our study, serovars reacted include serovars Pyrogenes, Pomona, Canicola and Hardjo among goats. In this study, higher seropositivity was noted for serovar Pyrogenes. In south Gujarat, serovars Pyrogenes, Hebdomadis, Autumnalis, Pomona and Grippotyphosa have been reported among rats [11]. This indicates rats might have been the source of infection to goats in this region.

No gross lesion of any pathological significance was observed in organs like kidneys, liver, spleen and lungs of seropositive goats. Histopathologically, in kidneys the changes were mostly confined to convoluted tubules while most of the glomeruli appeared apparently normal. The tubular changes comprised of varying degree of degenerative changes in lining epithelia and mild to severe degree of their desquamation (Figure 1). Some of the severely involved tubules were represented by basement membrane only. The lumina of convoluted tubules contained desquamated epithelial debris/casts with occasional erythrocytes. Regenerating tubules could not be observed in any section. Peritubular/periglomerular areas at places had mononuclear cell infiltration including lymphocytes and a few plasma cells (Figure 2). Stray foci of medullary congestion and oedema were also discernible. At places foci of fibroplasia were markedly seen (Figure 3). Modified Warthin Starry stained microsections in two goats showed structures indistinguishable from leptospires in some of the tubular lumina (Figure 4). In liver, varying degree of congestion was seen in central veins and sinusoids. Some of the portal areas exhibited mononuclear cell infiltration. The hepatic cords were disorganized/disrupted with individualization of hepatocytes and sinusoidal dilation (Figure 5). The affected hepatocytes showed varying degree of degenerative changes including fatty changes and focal areas of necrosis.

Histologically, in spleen mild to diffuse foci of hemosiderin pigment deposition, mild congestion and necrosis in the

central part of a few follicles were the characteristic features. Lungs revealed focal mononuclear cell infiltration in peri-bronchial/bronchiolar areas (Figure 6) and peribronchiolar fibroplasias. In addition, alveolar emphysema, focal areas of alveolar consolidation and edema were also discernible. Structures resembling leptospires could not be detected in the microsections of liver/lungs/spleen by Modified Warthin-Starry stain.

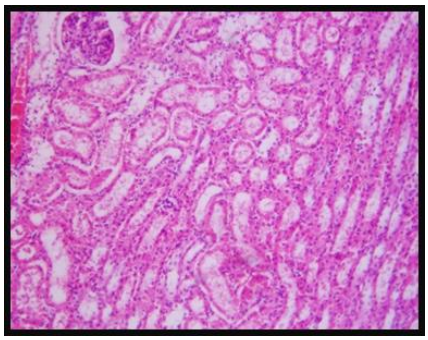

Fig. 1: Kidney: Degeneration and desquamation of tubular epithelium. Lower magnification. H&E x100

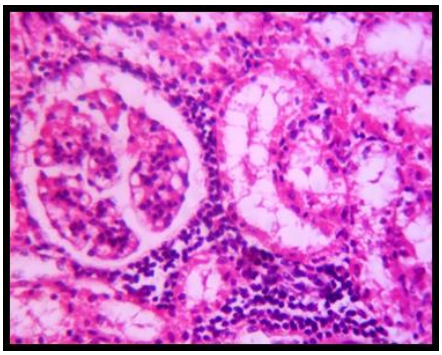

Fig. 2: Kidney: Peritubular and periglomerular mononuclear cell infiltration. H&E x400

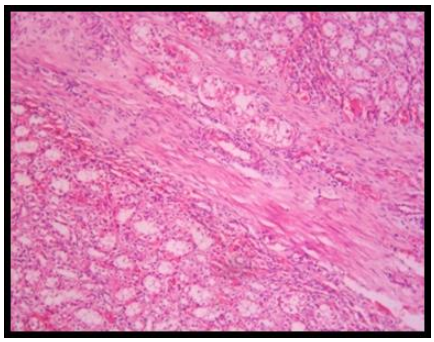

Fig. 3: Kidney: Fibroplasia. H&E x100

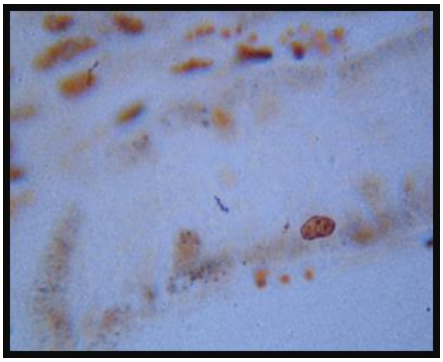

Fig. 4: Kidney: Structure resembling Leptospira in tubular lumen. Modified Warthin Starry Stain x1000

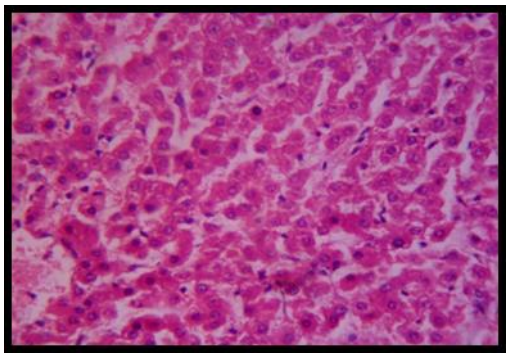

Fig. 5: Liver: Sinusoidal dilation and individualization of hepatocytes with mild degenerative changes. H&E x400

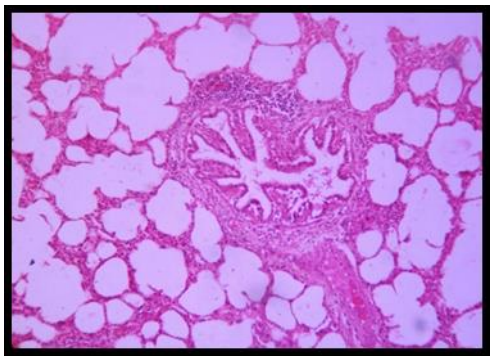

Fig. 6: Lung: Peribronchiolar mononuclear cell infiltration with emphysema. H&E x 100

In the present study no gross lesion was observed in any organ of seropositive animals. Almost similar observations were reported in cattle by Yener and Keles (2001) [12] who studied 68 white-spotted and 30 apparently normal kidneys in slaughtered cows for detection of leptospires using histopathology and immunohistochemistry. They found *Leptospira interrogans* antigen in 21/68 white-spotted kidneys and 4/30 grossly normal kidneys. Present findings supported the findings of earlier reports [13].

However, presence of white spots/foci on kidney surface among goats [13] and grayish white circumscribed foci/streaks measuring 1-4 mm in diameter with enlargement and pale brown appearance in sheep have been suggested a feature of leptospirosis by above workers. But these lesions are not specific for leptospirosis because comparable lesions occur in the kidneys of animal infected with septicemic colibacillosis, salmonellosis or brucellosis [14].

In the present study focal interstitial nephritis was seen only in one case which supported the findings of previous workers [13, 14, 15, 16] who mentioned that such lesions occurs in subacute/chronic leptospiral infection. Presently congestion was noted in kidney sections which supported the findings of previous workers [17].

Mild hemosiderosis, congestion and follicular necrosis noted in spleen supported the observations of earlier workers [13, 14, 15, 18]. Hemolysis results in the release of hemosiderin pigments in the spleen and would have been due to vasculitis with capillary injury [14]. Degenerative and mild inflammatory changes, congestion and periportal infiltration observed in the present study were in agreement with findings of previous workers [5, 7, 8, 17]. Further, pulmonary lesions observed in various cases supported the findings of some of the workers [15, 18].

Leptospires were seen in kidney sections of only two goats and supported the findings of many previous workers [5, 8] who

opined that leptospires are not always demonstrated in microsections impregnated with silver stain. The leptospires are either located within affected tubular epithelium or in tubular lumina. In past a number of workers demonstrated leptospires by silver impregnation method in different species of animals [19, 20] but demonstration of leptospires in microsections was always at low level.

Various pathological lesions observed in kidneys were suggestive of the fact that the kidneys are the site of predilection for leptospires as agreed upon by different workers world over. The hepatic and pulmonary lesions appear to be sequel of multiplication of the organisms and their spread during leptospiremic/acute phase [21]. With the development of antibodies leptospires starts their elimination from liver or other organs but its localization continues in kidney tubules [22].

The pathological changes observed in the present study in no way could be taken as specific for leptospirosis alone because leptospires, the etiological agent was detected only in a few cases in kidney tubules. The types of reaction observed were mostly degenerative and inflammatory in nature and may occur in a number of other infectious diseases like septicaemic colibacillosis, salmonellosis or brucellosis[14]. Morbid materials utilized in the study were from MAT positive/seropositive animals exclusively so we considered these pathological changes observed in various organs to be representative of leptospirosis.

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