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BILATERAL MIXED BACTERIAL PYELONEPHRITIS IN A CROSSBRED SHEEP

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ABSTRACT

Necropsy of a weaner, crossbred male sheep was performed having a clinical history of anorexia and weakness. The main finding revealed severe bilateral pyelonephritis. The right kidney was highly enlarged with patchy congestion, ruptured surface and haemorrhage. The renal pelvis and calyces were highly dilated with purulent content on sagittal section. The left kidney also had almost similar lesions. On bacteriological investigation *Corynebacterium pseudotuberculosis, C. renale and Proteus spp* were isolated. *C. pseudotuberculosis* was further confirmed by polymerase chain reaction (PCR), which yielded 551 bp and 382 bp amplified products of proline iminopeptidase (PIP), and nicotinamide adenine dinucleotide phosphate (NADP) oxidoreductase, respectively. Histopathologically, lesions were most severe in the medullary regions. The tubular lumen was dilated with densely eosinophilic casts. The glomeruli were less affected in comparison to the tubules. The interstitial tissues were infiltrated with lymphocytes and plasma cells. The renal pelvis was infiltrated with neutrophils.

Keywords *Corynebacterium pseudotuberculosis, Corynebacterium renale,* Necropsy, PCR, *Proteus*

Affections of the urinary system in sheep have been less commonly reported in comparison to other ruminant species. Amongst bacteria, *Corynebacterium pseudotuberculosis, Escherichia coli*, *Staphylococcus aureus, Actinomyces pyogens, C. renale and Proteus spp* have been found to be associated with urinary tract infections (Aiello and Mays, 1998; Radostits et al., 2000). A few earlier reports of bacterial infections of sheep urinary system consisted of infection with *C. renale* (Higgins and Weaver, 1981) and *E. coli* (Mahaffey, 1941) and an unnamed Gram negative coccobacillus (Fitch and Beaver, 1921). *C. pseudotuberculosis* has been found to be associated with the lesions/ abscessation in kidneys as a visceral manifestation of caseous lymphadenitis (Valli and Parry, 1993). *C. renale* colonizes lower urogenital tract of cattle and sometimes sheep (Hirsh et al., 2004). Pyelonephritis indicates inflammation of all parts of kidney involving the pelvis and parenchyma of the kidney. Infection in most of the cases is thought to be an ascending one from the lower region of the urinary tract. Although pyelonephritis is considered primarily a bovine disease, sheep are occasionally affected (Radostits et al., 2000).

MATERIALS AND METHODS

The study was carried out on a crossbred weaner male sheep (Patanwadi x GMM) carcass received from an organized farm of the Institute, situated in the semi-arid region of the Rajasthan. The animal had history of anorexia and weakness, without any specific diagnosis ante-mortem. The animal did not have any abnormality on external examination. Necropsy was conducted and changes in visceral organs were noted. Both kidneys were enlarged and showed suppuration on slicing. Pus and tissue samples were collected for microbiological, molecular and histopathological analyses.

For bacterial isolation, pus samples were directly inoculated on 5% defibrinated sheep blood agar plates followed by aerobic incubation at 37 ºC for 24 h. Based on the macroscopic characteristics of bacterial colonies and morphology in Gram's stained smears,

three types of colonies were identified and subcultured for further characterization (Quinn et al., 2011). All three isolates were tested for catalase, oxidase, urease, phospholipase D (PLD) and nitrate production and colony characteristics were studied on blood agar, potassium tellurite blood (5% v/v of 1% potassium tellurite), MacConkey agar and eosin and methylene blue agar (Himedia, Mumbai, India) (Cruickshank et al., 1975).

For isolation of the bacterial DNA, a few colonies from the three pure isolates on blood agar plates were transferred into 1.5 ml Eppendorf tube.The Bacterial DNA was extracted using HiPurA kit (Himedia, Mumbai, India) as per the manufacturer's instructions.The DNA was stored at -20º C until used. The bacterial isolates suspected for *Corynebacterium spp* were further subjected to molecular confirmation. Due to the prevalence of caseous lymphadenitis in the flocks from where this case originated, primers for two genes of *C. pseudotuberculosis* namely NADP oxidoreductase and PIP synthesized commercially (Sigma, USA) (Table 1) were used (D'Afonseca et al., 2010).

Table 1. The oligonucleotide primers for *C. pseudotuberculosis*

Target gene	Primers	Sequence (5'⟶3')	Length of PCR products (bp)
NADP oxidoreductase	Forward	ctg cga cat agc tag gca ct	382
	Reverse	ccg cca gac ttt tct cta ca	
Proline iminopeptidase	Forward	aac tgc ggc ttt ctt tat tc	551
	Reverse	gac aag tgg gaa cgg tat ct	

The DNA isolated from bacterial isolates was used as the template for PCR amplification. PCR was carried out in a final volume of 25 µl of reaction mixture containing 1xPCR buffer, 2 mM $MgCl_2$, 200 µM dNTP mix, 0.2 µM of each forward and reverse primers, 1.25 units of Taq DNA polymerase (Sigma, USA) and 5µl of DNA template. Amplification was performed in 200 µl tubes in a thermal cycler (MJ Research PTC200) through initial denaturation at 95 ^{0}C for 5 min was followed by 35 cycles each of 94 ^{0}C for 30 s, 54^{0}C for 45 s, 72^{0}C for 45 s and a final extension cycle at 72 ^{0}C for 5 min. A negative control without template and a positive control consisting of DNA from a characterized isolate were also included in the PCR. The PCR products were analyzed by visualization of desired size of DNA bands in the ethidium bromide stained agarose gel (2.5% w/v, 0.5X Tris borate EDTA buffer) under gel documentation system (Sambrook and Russell, 2001).

Following fixation of the pieces of the affected kidneys in 10% neutral buffered formalin, sections of the kidneys were trimmed and processed in graded alcohol, cleaned in xylene and embedded in paraffin wax. The sections were cut at 5 µm thickness and stained with haematoxylin and eosin (H & E) for histological examination (Culling, 1968).

RESULTS AND DISCUSSION

Both kidneys were highly enlarged, pale and soft in consistency and showed bilateral pyelonephritis. Urinary bladder and both ureters were distended with cloudy urine of alkaline pH of 8. The right kidney was highly enlarged (~ 7 times) and had shown patchy congestion, ruptured surface and haemorrhage (Plate 1a). The remaining portion of the kidney had irregular and elevated duffy surface. On careful sagittal section, the renal pelvis and calyces were highly dilated with pus (Plate 1b). Medullary crest were haemorrhagic and ulcerated. The cortex and medulla were thin and hemorrhagic at some places. The parenchyma was cystic and the capsule thickened. Similarly, the left kidney was enlarged (approximately 4 times) and had shown almost similar lesions.

Microscopically, lesions were most severe in the medullary regions. Proximal tubular epithelial cells at places showed degenerative and necrotic changes, which at times had revealed calcification. The tubular

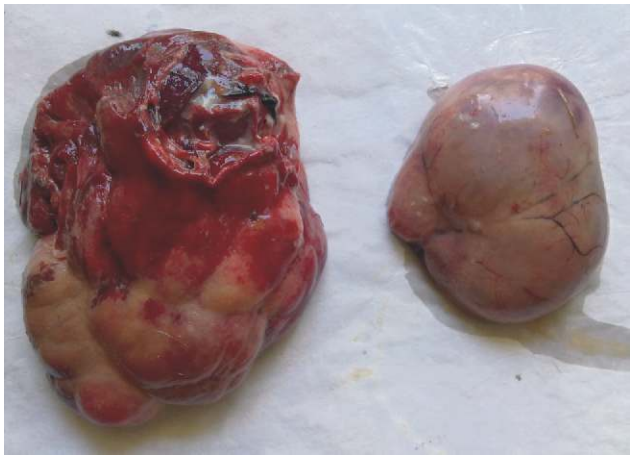

Plate 1a. Kidneys showing bilateral asymmetrical enlargement, soft in consistency and patchy congestion

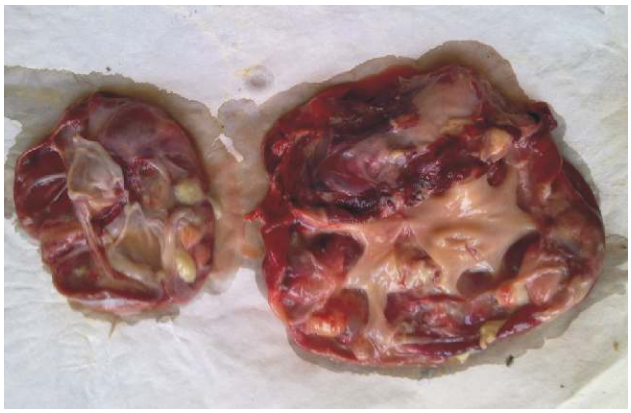

Plate 1b. Cut section of kidneys showing renal pelvis and calyces widely dilated with pus and haemorrhagic and ulcerated medullary crest

lumen was dilated with densely eosinophilic protein casts (Plate 2a). The necrotic debris were adherent to the denuded surface. The cortex was found compressed, the tubules were reduced in size and appeared as thin rim. The glomeruli were less affected in comparison to the tubules. The medulla of kidneys showed cystic spaces (Plate 2b). The periglomerular space was increased and some glomeruli were atrophied. The cystic spaces in the cortex were found to contain pale eosinophilic fluid and bacteria. The small arteries with thickened wall had eosinophilic material in their lumina. The interstitial tissues were infiltrated with neutrophils, eosinophils and plasma cells. Some blood vessels near pelvis were engorged with erythrocytes. The renal pelvis was highly dilated

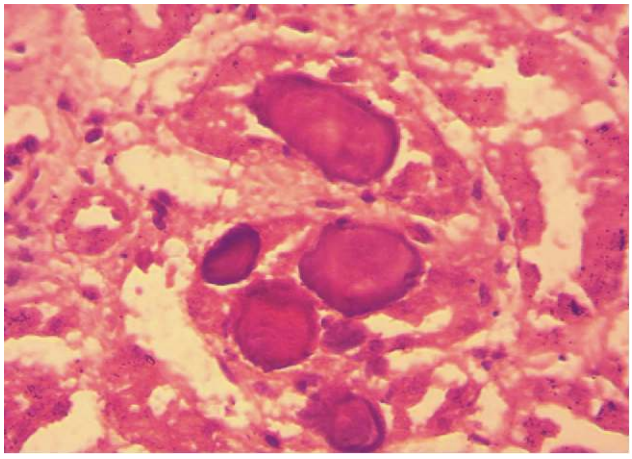

Plate 2a. Tubules showing necrosis of the epithelium, dilatation and contained densely eosinophilic protein casts (H & E x400)

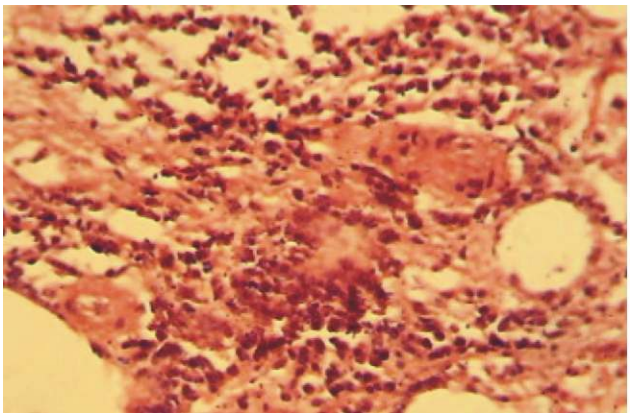

Plate 2b. Medulla of kidney showing cystic spaces (H & E x100)

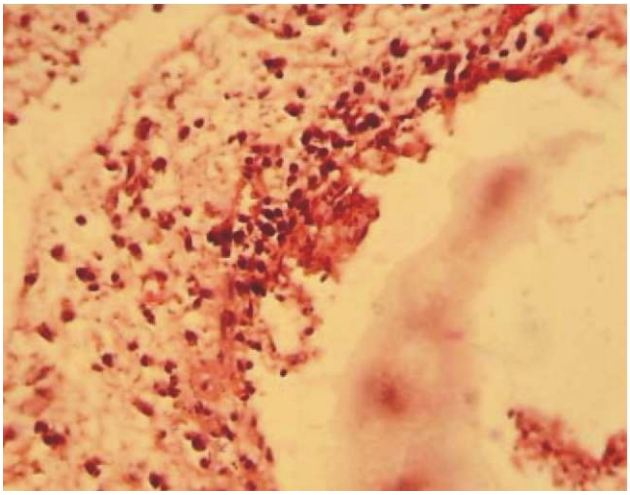

Plate 2c. The renal pelvis dilated with pinkish pale colour fluid containing clots of RBCs, fibrin and infiltrated with mononuclear inflammatory cells (H & E x400)

with pale colored fluid containing erythrocytes, fibrins and bacteria (Plate 2c). The renal pelvis was infiltrated with lymphocytes (Plate 2d).

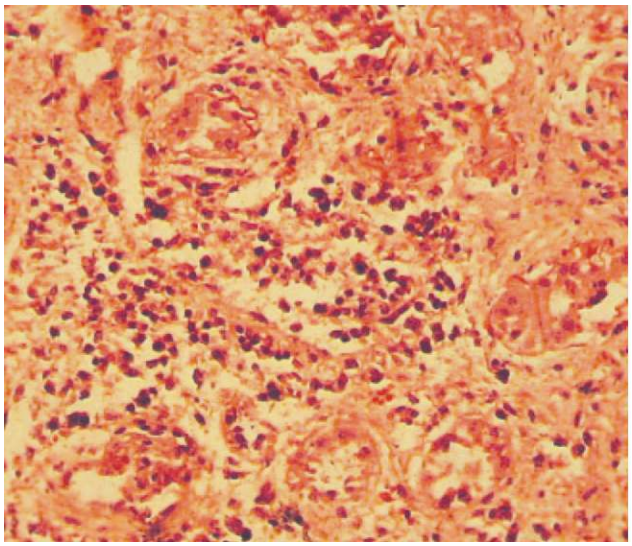

Plate 2d. Medulla of kidney showing mononuclear cell infiltration (H & E x100)

The colonies for bacterial isolate 1 were white-creamy, opaque, hemolytic (24 h) and convex on blood agar, which on staining were found to be Gram positive pleomorphic rods in various arrangements. On biochemical tests, they were catalase +ve, urease+ve, PLD +ve, nitrate-ve oxidase-ve, caseinase ve and non-lactose fermenters on MacConkey agar. The bacteria did not grow on EMB agar and colonies on potassium tellurite blood agar were black colored. The isolate on PCR yielded 382 bp and 551 bp amplified products of NADP oxidoreductase and PIP genes specific to *C. pseudotuberculosis* (Plate 3) (Quinn et al., 2011; D'Afonseca et al., 2010). The colonies for bacterial isolate 2 were yellowish-creamy, opaque, non-hemolytic and convex on blood agar. On staining, organisms were Gram-positive and diphtheroid /coccobacillary organisms in various arrangements. Biochemically, the isolate was catalase+ve, urease+ve, PLD-ve, nitrate-ve, oxidase-ve, caseinase+ve and non-lactose fermentor. The organisms did not grow on EMB agar. The colonies were black-colored on potassium tellurite blood agar. On molecular analysis, this isolate was negative for NADP oxidoreductase and PIP genes amplification. This isolate was identified as *C. renale* (Quinn et al., 2011).The colonies for bacterial isolate 3 were swarming, non-hemolytic and convex on blood agar. On staining, organisms were gram-negative and unusually longer bacilli. On biochemical tests they were catalase+ve, urease+ve, nitrate-ve, oxidase-ve, positive for H_2S production and non lactose fermentor. They did not grow on EMB agar. On molecular analysis, this isolate was also negative for NADP oxidoreductase and PIP genes amplification. This isolate was identified as *Proteus spp* (Quinn et al., 2011).

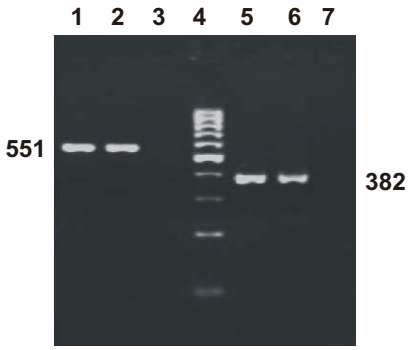

Plate 3. Identification of *C. pseudotuberculosis* isolate by PCR (Lane 4- molecular marker, Lane 1, 5- sample, Lane 2,6- positive control for respective gene, lane 3,7- negative control)

Out of various pathological conditions of kidneys, pyelonephritis has been less commonly reported in sheep (Jubb et al., 1993; Radostits et al., 2000). In a very few previous reports, ascending urinary tract infection due to *Proteus spp* has been rarely reported in small ruminants (Aiello and Mays, 1998). Therefore, various etiological agents responsible for suppurative infection in sheep have been rarely incriminated. The present report revealed a rare case of bilateral pyelonephritis in a crossbred sheep with a mixed infection of *C. pseudotuberculosis, C. renale* and *Proteus spp.*

The infection of kidneys with *C. pseudotuberculosis* has been infrequently described in sheep. This sheep did not exhibit any nodule on the cutaneous surface or in palpable superficial lymph nodes, and in any other visceral organs including lungs, liver, mammary glands, lymph nodes, etc, on necropsy and histological examination. Gross and histopathological findings in this case were similar to other reports (Jubb et al., 1993; Gyles et al., 2011). However, previous reports suggested ascending infection of the urinary tract with *C. renale* and *Proteus spp* (Hirsh et al., 2004; Caveney et al., 2011). Despite

the prevalence of clinical cases of caseous lymphadenitis in the flock and surrounding area, in this case presence of *C. pseudotuberculosis* infection without any detectable focus of infection appears peculiar. Though, infection with *C. pseudotuberculosis* in subclinical or dormant form and subsequent localization in any visceral organs can occur. Ascending infection along with *C. renale* and *Proteus* spp due to contaminated farm premises could be a possibility. Based on the results, it was difficult to pin point that which one of these three bacteria was a primary infection.

C. pseudotuberculosis has been reported to be associated with abscessation/lesion in visceral organs primarily the liver, kidneys or the mammary glands (Merchant and Packer, 1967). *Proteus* spp is indicated in UTI infection including pyelonephritis in small ruminants (Aiello and Mays, 1998). Involvement of diphtheroid organism indistinguishable from *C. renale* in pyaemic nephritis in a goat in India was reported (Dhanda et al., 1955). Although *C. renale* is the commonest cause of pyelonephritis in cattle there have been only occasional reports of pyelonephritis in sheep due to this organism (Gyles et al., 2011; Higgins and Weaver, 1981). In bovine, it has been reported that as the infection could be blood- borne, it is primarily an ascending one under natural condition and infected animals are known to be carrier of *C. renale* for a long period without clinical disease. In sheep, *C. renale* has also been reported to move from reproductive tract causing pyelonephritis (Quinn et al., 2011). Adherence of *C. renale* and other opportunistic uropathogens like Proteus spp to the urinary tract is a required virulence factor in the establishment of infection. Adhesion mediated attachment to urothelium and urea hydrolysis are considered critical in the pathogenesis of pyelonephritis by uropathogens (Quinn et al., 2011). Urea breakdown with production of ammonia initiates an inflammatory process, high alkalinity in urine and suppression of antibacterial defenses, possibly through complement inactivation. An inflammatory process successively involves the parts of urinary tract including renal pelvis and renal parenchyma resulting in pyelonephritis (Hirsh et al., 2004). Hemolysin produced by many uropathogens damages renal tubular epithelium and promotes invasive infection.

All the threes urease positive isolates had enough tissue damaging activity. It has been suggested that alkalinity associated with the liberation of ammonia from the urease- catalysed hydrolysis of urea may be, at least in part, responsible for the necrosis of the kidney tissue associated with pyelonephritis (Gyles et al., 2011). Since there was no contact with cattle directly but indirect contact through frequent entry of cattle and nilgai (*Boselaphus tragocamelus*) in the open grazing area of the farm or entry through replacement stock from field to maintain genetic variability could not be ignored. However, the source of infection could not be traced exactly. As in the cattle, some recent stress might have led to establishment of *C. renale* infection. In conclusion, the study involving different investigations revealed interplay of bacterial infection leading to the bilateral pyelonephritis in a sheep. This finding might add up to the understanding of the urinary tract infections of sheep.

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