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Case Report

Clinicopathological Aspects of the First Case of Bilateral Suppurative Pyelonephritis in Holstein Calves in Bulgaria

Ismet Kalkanov (*)

Trakia University, Faculty of Veterinary Medicine, Department of General and Clinical Pathology, 6000, Stara Zagra, Bulgaria

Abstract

The main objective of this study was to describe in detail the pathoanatomical and histopathological lesions found in the kidneys of three calves with bilateral suppurative pyelonephritis. The disease was observed for the first time in southeastern Bulgaria on a family cattle farm. The farm consisted of 48 adult Holstein cattle and 21 newborn and growing calves aged from 24 hours after birth to 45 days. The calves were born in the mothers' pens and then independently sucked colostrum. In the winter, within 72 hours, 5 calves fell ill and died: four females and one male, aged 5 to 9 days, with signs of dehydration, refusal of food and water, frequent and painful urination, pyuria, hematuria, kyphosis and fever. No treatment was given and no other calves in the group showed clinical signs. Three calf carcasses were submitted for necropsy and subsequent diagnostic testing by the farmer himself. After necropsy of the calves, tissue samples from the kidneys for histopathological examination. Samples of parenchymal organs (lung, liver, spleen and kidney), blood from the heart and a ligated section of the small intestine were also used for bacteriological studies. In conclusion, suppurative pyelonephritis is a rare multifactorial disease in newborn and growing calves with bacterial etiology. The macro- and microlesional changes in the kidneys of three calves described in the presented report will be of great benefit to veterinarians and researchers in the diagnosis, differential diagnosis and treatment of kidney diseases in ruminants.

Keywords: Bilateral suppurative pyelonephritis, calf, pathology, pathomorphology.

(*) **Corresponding author:**
Ismet Kalkanov
ismet_88@abv.bg

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Introduction

Morphogenetically, kidney lesions are classified as those affecting the glomeruli, renal tubules, interstitium, and renal pelvis. Some infectious agents damage nephrons, causing chronic renal lesions and leading to renal failure (Chou et al 2023). According to (Jarad et al 2020), renal diseases in ruminants often remain undiagnosed. There is a lack of sufficient data on the epidemiology of pyelonephritis in ruminants, which proves that the disease is not well studied, which makes its diagnosis and treatment difficult (Solomon et al 2020). Pyelonephritis and cystitis in cattle and calves are bacterial infections that cause inflammation of the urinary tract and renal parenchyma. Bacteria such as *Corynebacterium (C.) renale*, *C. pilosum*, *C. cystitidis*, *Escherichia coli* (*E. coli*), *Streptococcus* spp., *Proteus* spp. and *Staphylococcus* spp. are the causative agents of pyelonephritis and cystitis in cattle and calves (Constable 2022). *Mannheimia varigena* has been reported to cause pyelonephritis in growing Holstein calves (Komatsu et al 2019). Ascending infection with *C. renale* or *E. coli* in the urinary tract is the most common cause of pyelonephritis in ruminants. The hematogenous route is much less common and may result from bacteremia complicated by bacteria such as *Salmonella* species or *Actinomyces pyogenes* (Smith 2009, Nikkhah and Alimirzaei 2023). According to (Harada et al 2019), the most commonly studied *Mannheimia* is *M. haemolytica*, which is associated with diseases such as shipping fever, bovine respiratory disease syndrome, pyelonephritis and peritonitis in cattle. In the literature (Giannitti et al 2022) a case of *Bovine polyomavirus-1* (*BoPyV-1*, *Epsilonpolyomavirus bovis*) infection in cows with abortions is described. The fetuses had severe renal lesions caused by the virus, such as tubulointerstitial nephritis with cytopathic changes and necrosis in tubular epithelial cells, tubular and interstitial inflammation and interstitial fibroplasia. The authors of the report suggested that the lesions were

due to active viral replication in renal tubular epithelial cells that had intranuclear viral inclusions of *BoPyV-1*. Ultrastructurally, they also observed viral particles with typical polyomavirus morphology. Microscopic changes in the kidneys resembled the "cytopathic-inflammatory pathological pattern" of the pathogenesis of *Human polyomavirus-1* associated nephropathy in immunocompromised individuals and renal allograft recipients.

Due to the lack of sufficient information in the literature, the aim of this study was to describe in detail the pathoanatomical and histopathological lesions found in the kidneys of five Holstein calves in cases of bilateral suppurative pyelonephritis. It will make an application and contribution to the scientific and clinical literature, and will be of great benefit to veterinarians practicing with ruminants in the diagnosis and treatment of kidney diseases.

Case presentation

The owner's consent was obtained for the procedures undertaken and the use of the data for research purposes, his study was approved Trakia University Ethics Committee (105/2025). The reported case of bilateral pyelonephritis in adolescent calves, established on the basis of clinical, pathoanatomical, histopathological, and microbiological studies, was diagnosed for the first time on a family dairy cattle farm in southeastern Bulgaria. The farm consisted of 48 adult Holstein cattle and 21 newborn and growing calves aged from 24 hours after birth to 45 days. The animals were vaccinated preventively against mucosal disease - viral diarrhea, bovine herpes virus and respiratory disease complex. The animal hygiene conditions were poor, without a separate birthing room. The calves were born in the mothers' pens and then independently sucked colostrum. In the winter, within 72 hours, 5 calves fell ill and died: four females and one male, aged 5 to 9 days, with signs of dehydration, refusal of food and water, frequent and painful urination, pyuria, hematuria, kyphosis and fever. No treatment was given and no other calves in the group showed clinical signs. Three calf carcasses were submitted for necropsy and subsequent diagnostic testing by the farmer himself. After necropsy of the calves, tissue samples of 1 cm x 1 cm in size were obtained from the kidneys for histopathological examination and stained conventionally with hematoxylin-eosin (H/E). For antigen quantitative ELISA (BIOX Diagnostics, easy digest, Belgium) sandwich test for feces, *Rota, Corona*, *E.coli* F5, *Cryptosporidium parvum*. Intestinal contents from the necropsied calves and another 10 calves without clinical signs were used. Samples of parenchymal organs (lung, liver, spleen and kidney), blood from the heart and a ligated section of the small intestine were also used for bacteriological studies. As well as 20 ml of urine by bladder puncture in sterile containers for urine culture. During the macroscopic examination of the three carcasses of female calves, we found the following changes: bilateral enophthalmos, exhausted and dehydrated carcasses, stains in the area of the external genitalia with foul-smelling brownish urine with purulent traces. All three calves had purulent omphalitis and thrombosis of the umbilical blood vessels, and one calf also had local serofibrinous peritonitis. Numerous petechial hemorrhages were observed on the peritoneum, as well as on the wall of the urinary bladder, which was

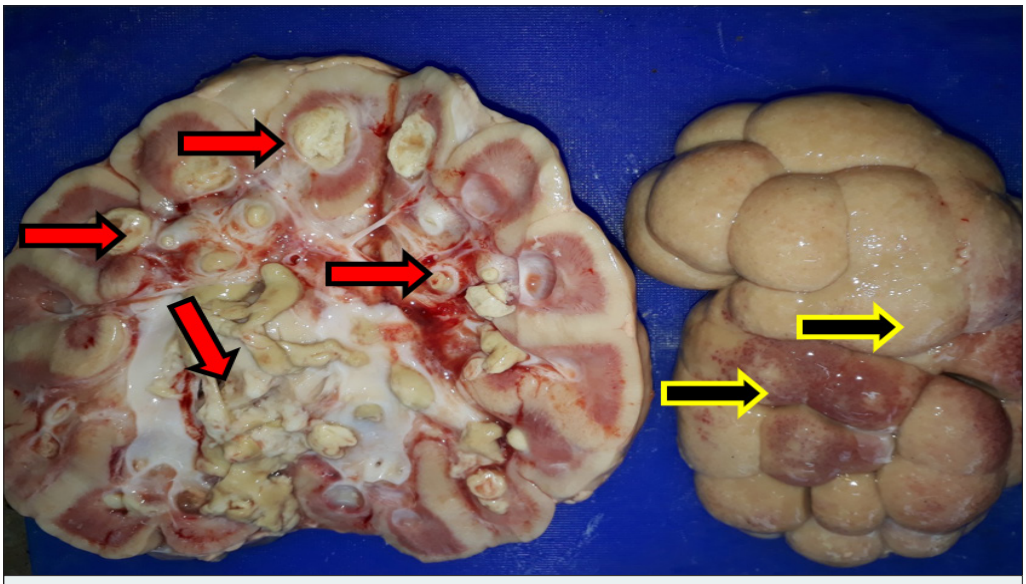

Figure 1. Enlarged and edematous kidneys with a pale color and subcapsular hemorrhages in the cortex (black arrows). On section, a pink-red cortical zone, pus in the pelvis and purulent-caseous foci in the parenchyma (red arrows). Calf with pyelonephritis

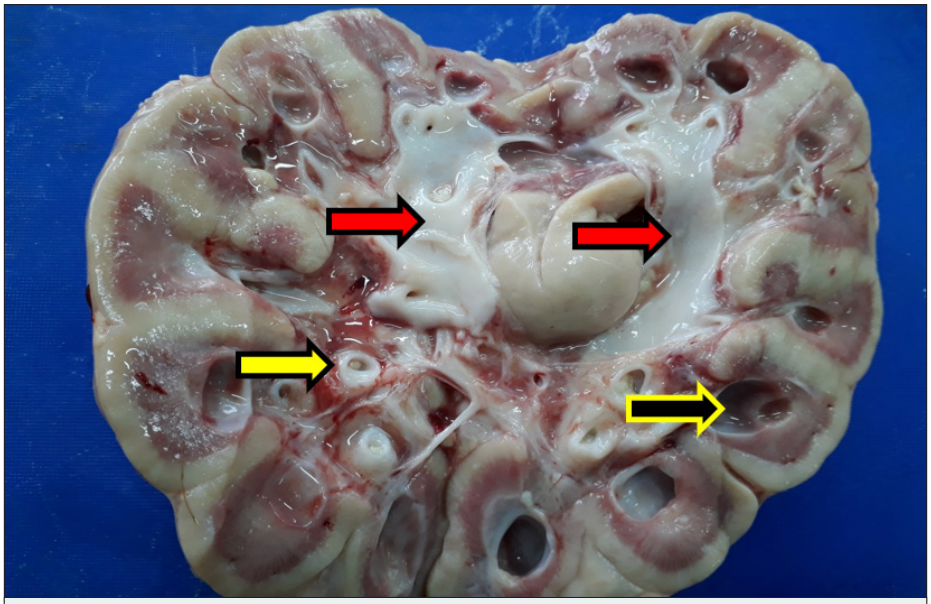

Figure 2. Fibrosis and dilatation of the renal pelvis (red arrows), papillary edema (yellow arrows), and diffusely scattered cavities (black arrows). Calf with pyelonephritis

filled with urine and greatly enlarged. The small and large intestines were filled with gases and pale yellow watery contents, and their mucosa was dotted with numerous punctate hemorrhages. The liver had a distended gallbladder. The kidneys in the three calves were enlarged with an edematous appearance, soft, flabby, crumbly and gelatinous consistency. The cortex was pale waxy, and numerous diffuse punctate hemorrhages and whitish nodules measuring 1 mm were visible under the capsule (Figure 1). The renal vessels were thrombosed, and the ureters were dilated with mucosal hemorrhages. On section of the kidneys, the pyramids were pink-red in color, the papillae were edematous, and the pelvis was severely dilated with edema, fibrosis, and single grains of sand mixed with abundant pus. Numerous purulent-caseous foci were observed in the cortex and medulla, forming oval cavities with a diameter of 10 mm, leading to compressive atrophy of the renal parenchyma (Figure 2). A team led by (Giannitti et al 2022) observed numerous punctate red and pale foci on the kidney cortex of a fetus

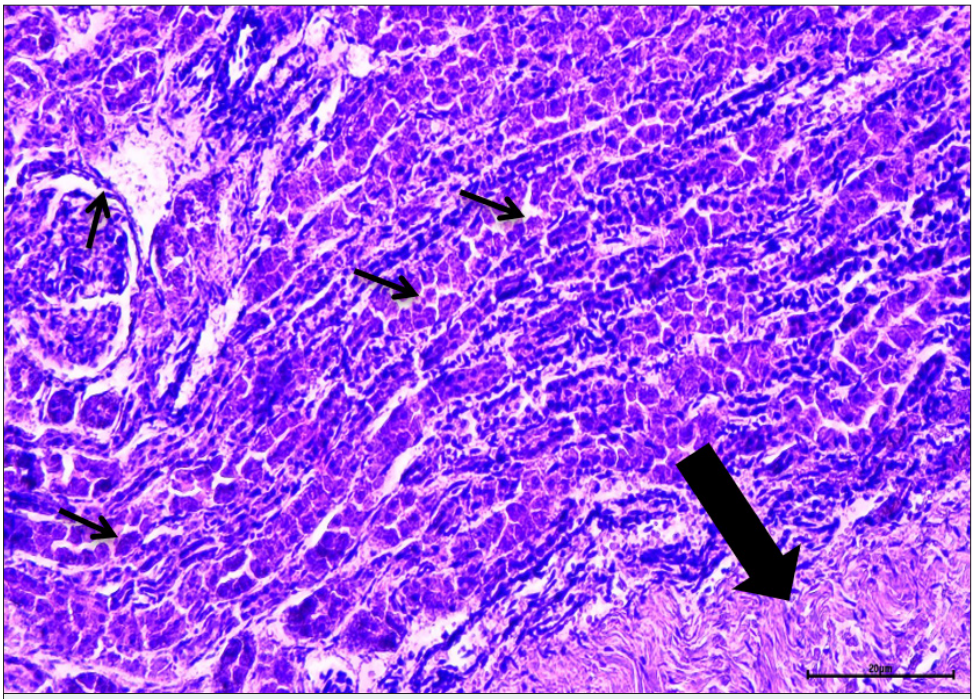

Figure 3. Degenerative-necrobiotic changes in the tubules and glomeruli (small arrows), inflammatory cell proliferations, hemorrhages and overgrown fibrous connective tissue (large arrow). Kidney, calf, H/E, Bar=10µm

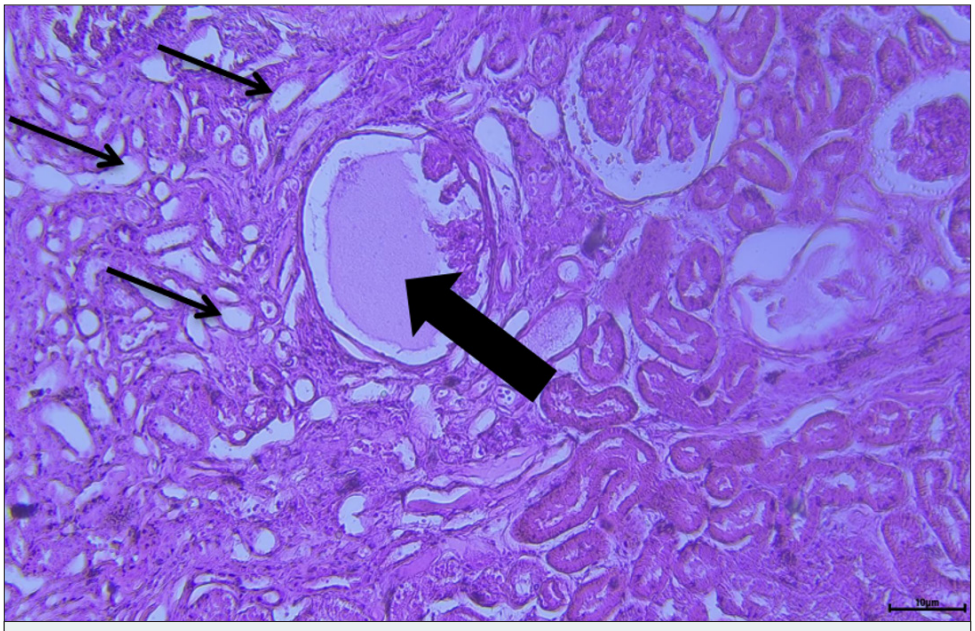

Figure 4. Necrotized and ectatic renal tubules (small arrows) exudate under Bowman's space (large arrow). Kidney, calf, H/E, Bar=10μm

infected with *Bovine polyomavirus-1*. On section, they reached the medullary part. In a section of the bladder, the urine was brownish, mixed with blood and purulent flocs. The mucosa was edematous, hyperemic, and hemorrhagic. However, no visible macrolesions were found in other organs. Microlesional changes in the kidneys represented multifocal degenerative necrobiotic changes in the tubules and glomeruli, inflammatory cellular elements, hemorrhages and overgrown fibrous tissue (Figure 3). In the cortical zone, the tubules were ectatic, containing hyaline droplets, degenerated neutrophils and necrotic epithelial cells. There was also tubular necrosis in individual tubules. In some areas there were also microabscesses composed of neutrophils. Cellular proliferates of histiocytes, plasma cells, lymphocytes and macrophages were observed in the interstitium. Serous glomerulonephritis with atrophy of the capillary body was also recorded (Figure 4). In individual glomeruli, the Bowman's capsule was thickened, some of the blood vessels were dilated and congested. In the renal pelvis, there was an increase in fibroblasts and fibrocytes - connective tissue. The results obtained from the laboratory etiological ELISA tests were positive only for *E. coli* K99 (F5) antigens in the intestinal contents of the three dead calves. During the bacteriological examination of the kidneys, liver and spleen of the three carcasses, *Corynebacterium pyogenes*, which causes urogenital infections in ruminants, was isolated and identified by colony morphology and Gram stain. *E. coli* was also isolated from the prepared urine culture. No other aerobic or anaerobic microorganisms were identified. A unique clinical case of pyelonephritis and cystitis in calves caused by *Corynebacterium pyogenes* and *E. coli* with 5% morbidity and 100% mortality diagnosed for the first time in Bulgaria. Complementing the epidemiology and etiology of the disease among the calf population. The literature so far lacks sufficient data on the pathomorphological changes in the kidneys in pyelonephritis in calves. The two pathogens identified by us in the etiological studies in the kidneys and urine, as well as coproantigens of *E. coli* K99 (F5), prove the presence of coinfection, the cause of high mortality in newborn and growing calves, as well as economic losses to farmers in the sector. Studies conducted by (Solomon et al 2020) in Israel in cows and calves with pyelonephritis and cystitis identified 65.9% of *E. coli* and 13.6% *Proteus mirabilis* in the urine. In contrast, we (Komatsu et al 2019) observed pyelonephritis in calves caused by *Mannheimia varigena* where the kidneys were reduced by multiple dark red lesions scattered throughout the cortex and hemorrhages in the renal pelvis. Other authors (Nikkhah and Alimirzaei 2023) describe a rare case of pyelonephritis with polycystic kidneys in a 5-day-old Holstein calf. Researchers such as (Mironova et al 2021) describe changes in the kidneys of calves with colibacillosis: juicy cut surface, dark red papillae. The liver was enlarged with a friable consistency, ochre-yellow in color, with hemorrhages of various sizes. In another study of ours (Kalkanov et al 2014) in calves with colibacteriosis, the umbilical vessels were thrombosed and the medullary zones of the kidneys were intensely hyperemic, giving them a cherry-red color. Similar microscopic changes to ours have been reported (Hailat et al 2023) in calves with leptospirosis nephritis: hyalinization, acute tubular

necrosis, and leukocyte infiltration. Komatsu and colleagues (Komatsu et al 2019) observed neutrophil infiltration, suppurative lesions, and hemorrhages in the renal parenchyma. Immunohistochemistry has detected *M. varigena* antigen in the cytoplasm of macrophages, neutrophils, and the interstitium of the renal cortex. Many years ago (Smith 1922) we observed lesions with a local character of lymphocytic infiltrates between the ectatic tubules in calves with interstitial focal nephritis caused by coli bacteria. In our previous studies (Kalkanov et al 2014), we reported acute serous nephritis in calves with colibacillosis. The renal tubules in the affected area were filled with serous fluid and hyaline casts. Authors (Giannitti et al 2022) described microscopic lesions in the kidneys of a fetus infected with *Bovine polyomavirus-1*. As a severe, widespread tubulointerstitial nephritis affecting the renal cortex and medulla. The affected tubules were ectatic with necrotic eosinophilic cell debris in the lumen. Multifocal tubular epithelial cells had vesicular nuclei containing pleomorphic basophilic viral inclusions, hypereosinophilic cytoplasm, and pyknotic nuclei. The interstitium was multifocally infiltrated with lymphocytes, histiocytes, plasma cells, neutrophils, and fibroblasts. Other authors, such as (Byndloss et al 2019) believe that bacterial urinary tract infections can occur in the early stages of calf life. They described congenital cysts in both kidneys of a calf caused by brucellosis infection. Since *Brucella* spp. attacks the trophoblast cells of the placenta, this can negatively affect the development of fetal organs. They also support the thesis that maternity ward hygiene and proper disinfection of the umbilical cord of newborn calves, along with optimal nutrition and welfare during the postpartum period, can be helpful in minimizing kidney infections in calves.

In conclusion, suppurative pyelonephritis is a rare multifactorial disease in newborn and growing calves with bacterial etiology. For its prevention, newborn calves should be raised in clean and disinfected premises, treated with a disinfectant on the umbilical cord, and given quality colostrum in a timely manner. The macro- and microlesional changes in the kidneys of three calves described in the presented report will be of great benefit to veterinarians and researchers in the diagnosis, differential diagnosis and treatment of kidney diseases in ruminants.

Declarations

Competing Interests
The author declared that there is no conflict of interest.

Availability of Data and Materials
The data that support the findings of this study are available on request from the corresponding author.

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Ethical Statement
Informed consent was obtained from the animal owner.

Author Contributions
Motivation / Concept: IK; Design: IK; Control/Supervision: IK; Data Collection and Processing: IK; Analysis and / or Interpretation: IK; Literature Review: IK; Writing the Article: IK; Critical Review: IK

ORCID
IK: https://orcid.org/0000-0003-2094-0369

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