Pyelonephritis caused by *Mannheimia varigena* in a Holstein calf

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**ABSTRACT.** A 7-day-old calf died following development of mild respiratory symptoms. Postmortem examination revealed the kidneys were inflamed, and Gram-negative bacteria was detected in the kidneys, supporting the diagnosis of suppurative pyelonephritis. *Mannheimia varigena* antigen was found in the lesions and the cytoplasm of macrophages and neutrophils in the renal cortex. The Gram-negative bacilli from the kidney were identified as *M. varigena* by sequencing the 16S rDNA. Although *M. varigena* is known to cause bovine respiratory disease syndrome, shipping fever, and meningitis, it was unknown that it could also cause suppurative pyelonephritis. Our study provides the first evidence of suppurative pyelonephritis caused by *M. varigena* in cattle and information that would improve our understanding, diagnosis, and treatment for *M. varigena* infections.

**KEY WORDS:** cattle, *Mannheimia varigena*, suppurative pyelonephritis

*Mannheimia* is a Gram-negative bacterium genus that encompasses *M. haemolytica*, *M. varigena*, *M. glucosida*, *M. granulomatis*, and *M. ruminantis* [3]. The most commonly studied *Mannheimia* is *M. haemolytica*, which is associated with diseases such as shipping fever, bovine respiratory disease syndrome [7], and peritonitis [9] in cattle. However, *M. varigena* infection is also associated with a number of conditions such as lung disease [2], shipping fever [10] and could also cause lesions such as suppurative meningitis [5], mastitis, endocarditis, and enteritis [3]. *M. varigena* is normally found within the oral cavity and the gastrointestinal tract [3], but could also be isolated from the upper respiratory tract of healthy calves [4] and the uterus of dairy cows [18]. Although *M. varigena* infection had been reported, there are few studies on histopathology and drug resistance studies using clinically isolated *M. varigena*.

Antibiotic resistance is an emerging global problem in livestock animals, and multi drug resistance had been reported in *M. haemolytica* [14] and drug resistance gene was not only *M. haemolytica* [14] but also *M. varigena* [12, 13], however, the information of drug resistance using clinically isolated *M. varigena* is limited.

Here, we report a fatal case of pyelonephritis caused by *M. varigena* infection, providing histopathological and bacterial characteristic of *M. varigena* in a neonatal calf.

A 7-day-old female Holstein calf at a farm housing 45 crossbred and 45 Holstein cattle was presented with rough breathing and fever (38.7°C) in January 2016. Despite ampicillin treatment, the calf died the next day. The animal was brought to Aichi Prefectural Chuo Livestock Hygiene Service Center where a postmortem necropsy was performed. *Mycoplasma bovis* was isolated from a nasal and ear swab of two calves that had died within a week of each other and no symptoms were detected in the adult cows and the other calves. Necropsy examination revealed that both kidneys were inflamed, and small, dark-red lesions were scattered throughout the renal cortex (Fig. 1a). Hemorrhage was found in the renal pelvis on the cut surface of the kidney. Furthermore, the lungs showed dark red lobules. However, no visible lesions were found in the other organs. Tissue samples of the liver, spleen, left kidney, heart, lung, thymus, and brain were fixed in 10% neutral-buffered formalin. Fixed tissues were embedded in paraffin wax, sectioned at approximately 3 µm thickness, and stained with hematoxylin and eosin (H&E) or Gram stain for histological examination. To
label the *M. varigena* antigen, 3-µm thick sections of formalin fixed samples of liver, spleen, kidney, and lung were treated with 3% hydrogen peroxide in methanol to suppress endogenous peroxidase activity. Antigen retrieval was performed by treating the sections with 0.1% actinase E solution in phosphate buffered saline at 37°C for 20 min. The tissues were then incubated with rabbit anti-*M. varigena* 971 strain serum (National Institute of Animal Health, Tsukuba, Japan) as primary antibody for 60 min at room temperature followed by a secondary antibody (Histofine Simple Stain MAX-PO (Multi; Nichirei Bioscience Inc., Tokyo, Japan) for 30 min at room temperature. The sections were then incubated with aminoethyl carbazole (AEC) substrate solution (Histofine Simple Stain AEC solution; Nichirei Bioscience Inc.) at room temperature for 5 min. and then counterstained with hematoxylin. Sections of tissues containing *M. varigena* and pieces of liver (into which *M. haemolytica* serotypes A1, A2, A5-A9, A12-A14, A16, *Bibersteinia trehalosi* serotypes T3, T4, T10, T15 and *M. glucosida* had been injected) were used as positive controls in order to verify the immunohistochemical specificity of the reaction. *Pasteurella haemolytica* biotype A had 13 serotypes and *P.
haemolytica biotype T had 4 serotypes [16]. Subsequently, in 1999, based on the results from DNA-DNA hybridization and 16S RNA studies, all serotypes of *P. haemolytica* biotype A were grouped under a newly created genus *Mannheimia* [16]. All serotypes under biotype A became *M. haemolytica* except A11, which became *M. glucosida*. All four serotypes of *P. haemolytica* biotype T were named as *P. trehalosi* retaining the genus *Pasteurella* [16]. Further taxonomical analysis in 2007 resulted in the creation of a new genus, *Bibersteinia*, under which were included all the four *P. trehalosi* serotypes, later named as *B. trehalosi* [16].

Suppurative lesions were widely detected in the renal pelvic mucous membrane and renal papilla (Fig. 1b) and showed numerous neutrophils and oast-like cells (Fig. 1c). Gram-negative bacilli were detected in the lesions and the lumen of the renal pelvis.

Moderate to severe congestion and hemorrhage were observed surrounding the suppurative lesion in the inner medulla (Fig. 1b), and thrombi were also scattered in the lesions. Cellular debris and neutrophils were found in some of the collecting and renal tubules; the neutrophils had also infiltrated the interstitium surrounding the tubules. In other organs, thymocytes were decreased in the thymus cortex, and neutrophils were found around the white pulp of the spleen. Slight edema and congestion were observed in the lung. No significant lesions were detected in other organs. Gram-negative bacteria with *M. varigena* antigens were detected in the suppurative lesion and lumen of renal pelvis (Fig. 1d) and cytoplasm of macrophages and neutrophils in the interstitium (Fig. 1e). No antigen was detected in the liver, spleen and lung. In addition, a positive reaction was detected only in the positive control sections of tissues containing *M. varigena*, but not in the other positive controls. The rabbit anti-*M. varigena* 971 strain serum specifically reacted with *M. varigena*, but not in the other positive controls. The rabbit anti-*M. varigena* 971 strain serum specifically reacted with *M. varigena*. To determine the susceptibility of the *M. varigena* to antibiotics, minimum inhibitory concentration (MIC) was calculated using broth dilution method according to clinical laboratory standards institute (CLSI) method [8] against ampicillin (ABPC), ceftiofur (CTF), tetracycline (TC), florfenicol (FF), enrofloxacin (ERFX), and danofloxacin (DNFX). The breakpoints of these antibiotics were referred from the breaking point of *M. haemolytica* based on CLSI [8] and Alexander et al. [1]. The isolate analyzed in the current study was susceptible to all antimicrobial drugs (Table 1).

Our results confirmed *M. varigena* infection in the dead Holstein calf, which could be the cause of the suppurative lesions in the renal pelvis. *M. bovis* was not detected in the lung homogenates, and therefore, it is unlikely to be associated with the pulmonary lesions. Although *M. varigena* could cause various lesions such as suppurative meningitis [5], mastitis, endocarditis, enteritis [3], and pneumonia [2], it is unknown if *M. varigena* is associated with pyelonephritis, and therefore, this is the first report of pyelonephritis caused by *M. varigena*.

In the current study, suppurative lesions and bacterial isolation were limited only to the kidney. Gram staining and histological examination revealed that *M. varigena* was present only in the suppurative lesions of kidney. Although the bladder or ureter were not examined, *M. varigena* would be infected via urethra because of limited detection of *M. varigena*.

In our case, the isolates were misidentified as *M. haemolytica* by commercial biochemical test. However, the 16S rDNA profiles differ between the different *Mannheimia* species [11] and we determined the isolated bacteria to be *M. varigena* using this method. It was reported that *M. varigena* is the most frequently isolated bacteria from bovine respiratory disease after *M. haemolytica* [17], and reliable detection methods for *M. varigena* such as PCR would be useful for accurate and rapid diagnosis.

Drug resistance is one of the biggest concerns in raising livestock. Although tetracycline [13] and multi-drug resistance genes

<table>
<thead>
<tr>
<th>Class</th>
<th>Antimicrobial agents</th>
<th>MIC</th>
<th>Breaking point</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>Ampicillin (ABPC)</td>
<td>≤1</td>
<td>16</td>
<td>[1]</td>
</tr>
<tr>
<td>Cephe</td>
<td>Ceftiofur (CTF)</td>
<td>≤0.12</td>
<td>8</td>
<td>[8]</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Tetracycline (TC)</td>
<td>0.5</td>
<td>8</td>
<td>[8]</td>
</tr>
<tr>
<td>Amphenicol</td>
<td>Florfenicol (FF)</td>
<td>≤1</td>
<td>8</td>
<td>[8]</td>
</tr>
<tr>
<td>New Quinolone</td>
<td>Enrofloxacin (ERFX)</td>
<td>≤0.03</td>
<td>2</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>Danofloxacin (DNFX)</td>
<td>≤0.03</td>
<td>0.25</td>
<td>[8]</td>
</tr>
</tbody>
</table>

MIC: minimum inhibitory concentration.
had been detected previously in *M. varigena* [4], the isolate in this study did not show drug resistance to any of the tested drugs. Although the calf in this study was treated with ampicillin immediately following presentations of breathing difficulties, the symptoms were not alleviated despite lack of resistance to ampicillin. This could be explained in part by the fact that kidney functions were attenuated beyond recovery before ampicillin treatment. Therefore, it is important that diagnosis is made rapidly in these situations.

In conclusion, we have reported a unique case of pyelonephritis caused by *M. varigena* in a calf. Although *M. varigena* could cause a number of diseases, pyelonephritis had not been associated with *M. varigena* infection. *M. varigena* could be misidentified by using commercially available biochemical test and 16S rDNA sequence analysis is required to correctly identify *M. varigena* infection. This study provides evidence that *M. varigena* could cause pyelonephritis and correct identification of the infectious agent and drug resistance would be useful for future treatments.

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REFERENCES


