Bovine Stillbirth Due to Nocardia farcinica

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ABSTRACT. A stillborn calf at 259 days of gestation was examined. The dam showed no clinical signs of disease, and the stillbirth was occurred sporadically and were characterized by focal necrosis surrounded by infiltration of the cells such as macrophages and multinucleated giant cells. The lesions were observed systemically. The hyphae were visible by Grocott’s stain and they were positive by immunohistochemical stain using serum of a rabbit immunized with the isolated organism. The isolated bacteria were determined to be Nocardia farcinica by bacteriological and molecular analysis and we confirmed that the stillbirth was caused by infection with and proliferation of Nocardia farcinica. This is the first report of a bovine stillbirth caused by this organism.

KEY WORDS: cattle, Nocardia farcinica, stillbirth.

Nocardia species are common soil saprophytes that are ubiquitous in the soil and water and on plants. They cause infection by wound contamination, inhalation, or ingestion. They are aerobic, gram-positive, filamentous organisms that may be acid fast [13]. Pulmonary, systemic, and central nervous systems nocardiosis have been reported in humans [1]. Brain abscess [17] and psoas abscess [18] related to Nocardia farcinica infection have also been reported in humans. Nocardiosis in animals has often been reviewed [16, 20] including reports of mastitis in cattle [4], and in goats [7] and bovine farcy [10, 11]. A few cases related to nocardial abortion have been reported in cattle [12, 22] as well as in horses [3], pigs [9], and sheep [22]. The organism initially placed in the genus Nocardia was isolated in 1888 from a case of bovine farcy (lymphadenitis) [15]. One year later, the organism was characterized and named Nocardia farcinica. Since that time, the taxonomic history of the genus Nocardia has been fraught with confusion and controversy. It had been characterized as a Mycobacterium strain or N. asteroides. However, with the application of methods including susceptibility testing and molecular studies, the species status of N. farcinica was clearly established recently as separate from N. asteroides [5, 13].

A calf was stillborn on a farm raising 11 cows and 5 calves. Clinical signs of disease were not observed in the dam before or after expulsion of the fetus, and no significant disorders were detected in the other cows at the same farm or in surrounding area. The stillborn calf was Japanese Black beef cattle, at 259 days of gestation and was necropsied for diagnosis. The brain, heart, lung, liver, kidney and spleen were collected and submitted to microbiological culture at a temperature of 37°C using 5% sheep blood agar (aerobic, anaerobic), chocolate agar (air plus 5% CO₂) and DHL agar (aerobic). After 1-week incubation under aerobic and air plus 5% CO₂ conditions, light yellow colonies were observed from all the organs except the brain. The isolate was Gram-positive, polymorphic bacilli containing mycolic acid and was partially acid-fast by Ziehl-Neelsen stain, suspicious for Nocardia sp. The organism was positive for catalase, urease, β-lactamase, acid production from rhamnose, and growth at 45°C, but negative for oxydase and hydrolysis of casein, xanthine, hypoxanthine and adenine. In order to firmly identify the isolate, the 16S rDNA gene sequence was determined, as described previously [14], and compared with all bacterial sequences available from DDBJ/EMBL/GenBank databases. The determined sequence (1,420 bp; DDBJ/EMBL/GenBank accession no. AB490157) showed 100% homology with that of Nocardia farcinica type strain DSM 43665 (DDBJ/EMBL/GenBank accession no. AF430033). On the basis of the result, together with the morphological, biochemical, and culture characteristics, we identified the isolates as Nocardia farcinica. For pathological examination, tissue samples were taken from the brain, internal organs and lymph nodes of the stillborn fetus. The placenta was not available for examination. Samples were fixed in 10% neutral-buffered formalin, processed routinely and embedded in paraffin wax. Sliced sections were stained by haematoxylin and eosin (HE) for routine light microscopic examination. Additionally, Gram stain, Grocott’s stain, and Ziehl-Neelsen stain were also performed for pathogen detection. Immunohistochemical (IHC) labeling for detection of the causative agent within lesions was performed with a commercial kit (Histofine SAB-PO kit, Nichirei, Tokyo, Japan). A rabbit antiserum against the isolated bacilli was used as the primary antibody. Briefly, the isolated bacilli were inactivated with 0.5% formalin-saline solution and washed were inoculated a rabbit intravenously twice a week for 1 month [6]. The antiserum was made from the blood collected from the rabbit under anesthetic condition. Macroscopically, congestion of the cerebral meninges was observed. The lung was hardened.

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with necrotic foci accompanied by hemorrhages. Necrotic foci were also observed in the liver. Erosions were seen at the mucosal surface of the abomasums, and multiple necrotic foci seen as white nodules were observed at the mucosa (Fig. 1). In the small intestine, multifocal necrotic foci were found at the serosa and hemorrhagic foci and/or erosions were seen at the mucosal membrane. The mesentery had an edematous appearance. Superficial and abdominal lymph nodes were also edematous and often had necrotic foci. Enlargement of the mesenteric lymph nodes was prominently observed. Major microscopic lesions were characterized by focal necrotic changes (Table 1) and these lesions were observed in most of the organs examined. By microscopic examination, congestions were confirmed the macroscopic findings. In the lung, multifocal coagulation necrosis was prominently observed, and it was surrounded by edema, hemorrhage, infiltration of macrophages, multinucleated giant cells, and epithelioid cells, i.e., granulomatous lesions (Fig. 2A, B). A number of Gram positive, polymorphic bacilli were found at the necrotic foci. Multifocal necrotic lesions were also observed in the liver, spleen, kidney, lymph node, thyroid, adrenal and tongue. Mucosal erosion and focal necrosis were observed in the lamina propria and submucosa of the omasum and abomasum. Necrotic foci accompanying the organisms were also observed in the submucosa of the small intestine. In the central nervous system, especially the cerebrum (parietal lobe, occipital lobe), midbrain, choroid plexus, pons and pars cervicalis, showed suppurative encephalitis and necrosis accompanying the multifocal perivascular gliosis. Suppurative meningitis was also seen in the cerebrum (parietal lobe), cerebellum and medulla oblongata. A number of hyphae were observed in most of the necrotic foci distributing systematically. They were stained black with Grocott’s stain (Fig. 2C). The organism was partially stained by Zeil-Neelsen stain after the sections were deparaffinized with benzene and decolorized with 10% sulfuric acid. Positive reactions were detected in the necrotic foci relating to the organisms by IHC (Fig. 3A, 3B).

In dairy animals, especially cows, mastitis is a distinct clinical manifestation of major importance. Large numbers of outbreak of nocardial mastitis have been reported in the world [8] and abortions following nocardial infection have been reported in several animal species [3, 9, 12, 21, 22]. The specific routes of these infections are not clearly understood, but it has been suggested that contamination of the genital system, especially during obstetrical manipulations, may lead to uterine infection [19]. This in turn may result in necrotic lesions on the chorionic surface of the placenta leading to abortion of fetus. In addition, it has been reported that fetus can become heavily infected, and dissemination of the disease throughout the fetus result in abortion. The inci-
dence of bovine abortion caused by *Nocardia* is not known, but it is probable that many other agents have been incorrectly identified as the etiology of *Nocardia*-induced abortion. Therefore, misdiagnosis has almost certainly resulted in an underestimate of the importance of *Nocardia* in abortion in cattle [2]. In the present study, systemic necrotic granulomatous lesions were observed in the stillborn calf and the causative agent was confirmed as *Nocardia farcinica* by the bacteriological examination. The lesions were distributed in various organs such as the lung, digestive system, lymph nodes and central nervous system. It is likely that the organisms invading the uterus were proliferated and were disseminated over the fetal organs via the amniotic fluid or fetal circulation, and resulted in necrotic-granulomatous lesions and induced stillbirth. The dam did not show any clinical signs and similar abortion/stillbirth had not occurred in the cows raised on the same farm. Therefore, the case observed in this study was thought to be a sporadic incidence. This is the first report of bovine stillbirth caused by *Nocardia farcinica*.

REFERENCES


Table 1. Distribution and the grades of the lesions observed in this study

<table>
<thead>
<tr>
<th></th>
<th>Necrosis</th>
<th>Cell infiltration</th>
<th>Detection of hypha by Grocott stain</th>
<th>Other lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mononuclear cell</td>
<td>Neutrophil</td>
<td></td>
<td>Infiltration of multinuclear giant cell</td>
</tr>
<tr>
<td>Lung</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>Liver</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>Heart</td>
<td>–</td>
<td>+</td>
<td>±</td>
<td>NT</td>
</tr>
<tr>
<td>Kidney</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>Spleen</td>
<td>+</td>
<td>++</td>
<td>±</td>
<td>None</td>
</tr>
<tr>
<td>Abomasum</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>Jejunum</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>None</td>
</tr>
<tr>
<td>Colon</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>Tracheobronchial lymph node</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>Perivascular inflammation, Meningitis</td>
</tr>
</tbody>
</table>

+++: severe lesions were diffusely distributed. ++: moderate lesions were multifocally observed. +: slight lesions were rarely observed. ±: few cell infiltrations were observed. NT: not tested.


