**Fibrinonecrotic Rhinitis Caused by a Concurrent Infection of *Fusobacterium necrophorum* and *Arcanobacterium pyogenes* in a Cow**

Yukio M. SEIMIYA1), Maki TAKAHASHI1), Takashi TAMURA1), Ryukoh MURAKAMI1), Makoto HARITANI2) and Kumiko M. KIMURA2)


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**ABSTRACT.** An 8 year-old cow showing severe dyspnea and nasal mucosal necrosis immediately after parturition was subjected to pathological examination. The principal lesions were fibrinonecrotic rhinitis, necrotic bronchopneumonia and renal infarction. *Fusobacterium necrophorum* biotype A and *Arcanobacterium pyogenes* antigens were detected in the nasal and pulmonary lesions. These results suggest that the lesions were caused by a concurrent infection of the detected bacteria and that the pulmonary lesions were caused by the aspiration of infectious materials from the nasal ones. Mucosal coagulative necroses observed as the initial lesions in rhinitis were frequently associated with multiple thrombosis. The findings might suggest that thrombosis played an important role in the development of the nasal lesions.

**KEY WORDS:** *Arcanobacterium pyogenes*, fibrinonecrotic rhinitis, *Fusobacterium necrophorum*.

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*Fusobacterium necrophorum*, a normal inhabitant of the alimentary tract of animals and humans [15, 17], is an opportunistic pathogen that causes necrotic conditions such as bovine hepatic abscesses [9, 15], interdigital necrobacillosis [1, 7], caudal vena caval thrombosis [4], glossitis [16, 20] and calf diphtheria [10]. However, there are few reports on bovine rhinitis associated with the bacterial infection. Although the pathogenic mechanism of *F. necrophorum* infection remains unclear and is clearly multifactorial, several toxins are suggested to play a role in the establishment of infection or subsequent lesional development [3, 6, 11, 19]. Synergisms between *F. necrophorum* and other bacteria are also associated with the mechanism [8, 11–13, 18], since *F. necrophorum* is frequently encountered in concurrent infections in which *Arcanobacterium pyogenes* is most commonly involved [5, 7, 9, 14, 15, 20]. This study describes the pathological findings of a dairy cow suffering from fibrinonecrotic rhinitis with the detection of *F. necrophorum* and *A. pyogenes* antigens.

An 8 year-old cow was immediately affected after parturition, on a farm that was raising 15 Holstein-Friesian cow or calves located in Iwate Prefecture, Japan. The cow showed difficulty in standing and recovered after the administration of calcium solution. However, the cow became anorectic after parturition on the same day. The clinical condition worsened day by day, followed by tachypnea on the 2nd day and pyrexia of 39.5°C on the 3rd day. Yellowish mucosa with mucopurulent discharge and stenotic sound were present on the 4th day, although ampicillin and electrolyte were administered on the 3rd and 4th days. The cow was euthanatized because of severe dyspnea on the 7th day.

Macroscopically, the mucosa was yellowish throughout the bilateral nasal passages, with fibrinous exudate mingling with blood and being difficult to remove (Fig. 1). The exudate in the mucosa of the vestibular region was rich in mucous elements. The nasolabial corneal layer was thickened and partly sloughed resulting in an irregular surface. Yellowish lesions measuring 1 to 2 cm in diameter were present sporadically in the bilateral cranial and middle pulmonary lobes, and discolored lesions, the size of soybean, were found in the bilateral renal cortical areas.

Tissue blocks collected from the whole body, including liver, spleen, kidneys, heart, nasal mucosa, trachea, pulmonary lobes, adrenals, pancreas, submaxillary, parotid and thyroid glands, tongue, alimentary tract, skeletal muscles, urinary bladder, gallbladder, tonsils, lymph nodes, thymus, cerebrum, spinal cord and peripheral nerves, were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections of tissue were stained with hematoxylin and eosin (HE). Selected sections of the nasal mucosa, pulmonary lobes and kidneys were stained with phosphotungstic acid in lead citrate (PTA) stain for transmission electron microscopy (TEM).

Fig. 1. Necrotic nasal mucosa (left side) is covered by fibrinous exudate with hemorrhages (right one).
acid hematoxylin (PTAH) and Gram as well as by the von Kossa’s and streptavidin-biotin-peroxidase (SAB) methods. The SAB method was applied using a SAB kit (Nichirei Corporation, Tokyo). Rabbit hyperimmune sera against *F. necrophorum* biotype A, *A. pyogenes*, *Pasteurella multocida* types A, B, D and E and parainfluenza 3 virus or mouse monoclonal antibodies against bovine respiratory syncytiat (ARGEN, France) and infectious bovine rhinotracheitis viruses (VMRD, U.S.A.) were used. The reaction products were visualized with 3,3’-diaminobenzidine tetrahydrochloride (DAB) in Tris-buffered saline. Sections were counterstained with methyl green. The specificity of each antiserum was confirmed using bovine hepatic or pulmonary tissues that were experimentally inoculated. Negative control slides were stained in the absence of primary antibodies.

The principal histological changes were found in the nasal mucosa, pulmonary lobes and kidneys. There were various developmental stages of the lesions in the nasal mucosa. The initial lesions were characterized by laminar coagulative necrosis of the superficial mucosa and multiple thrombosis. Necrotic areas involving the mucosal epithelial layer and the underlying lamina propria were clearly discriminated from the other regions by a zone of inflammatory cells. Congestion, edema, fibrinous thrombi of many capillaries and venules and several infiltrated neutrophils and macrophages were found in the necrotic areas (Fig. 2A). The lesions were followed by pronounced fibrinopurulent exudation, hemorrhages and sloughing of the necrotic areas.

In more advanced lesions, the naked lamina propria was covered with regenerated mucosal epithelia, often derived from the neighbouring nasal glandular ducts. Infiltration of lymphocytes, plasma cells and fibroblasts was present in the lamina propria (Fig. 2B). Numerous bacterial clumps were found on the surface of the necrotic areas or in fibrinopurulent exudate present in the lamina propria and the nasal lumen.

In the deeper mucosa, the epithelia of the nasal glandular acini were hyperexcrective, occasionally degenerative or necrotic. The nasal glandular ducts were distended and filled with sero-mucin and infiltrated neutrophils and macrophages.

Multifocal necroses were observed in the pulmonary lobes. The lesions comprised a central area of coagulative necrosis with bacterial clumps and a surrounding dense zone of inflammatory cells (Fig. 3). Alveol and bronchioli adjacent to the necrotic lesions contained large amounts of fibrinous exudate, infiltrated neutrophils, macrophages and lymphocytes, and occasionally multinucleated syncytiat cells. Sero-fibrinous exudate was seen in the thickened interlobular tissue.

Infarcts were present in the renal cortical areas. The lesions were wedge-shaped and accompanied by hyalino ux degenerated walls and fibrinous thrombi of the interlobular arterioles. Fibrinous thrombi were also found in several glomerular capillaries in the necrotic lesions. Coagulative necroses were observed in the proximal and distal tubuli and glomeruli in the central zones of the lesions, and

Fig. 2. Nasal mucosa. A) The initial lesion is characterized by mucosal laminar coagulative necrosis surrounded by a zone of inflammatory cells and multiple thrombosis (arrow heads). B) The necrotic area desquamates with pronounced fibrinopurulent exudation (left side), and the naked lamina propria is covered with regenerated mucosal epithelia (central one). HE stain, × 60. C and D) Numerous *F. necrophorum* (C) and *A. pyogenes* (D) antigens are present in the extracellular space or intracytoplasm (arrow heads) of inflammatory cells in the necrotic areas. SAB method, × 580.
in the proximal tubuli in the marginal zones. Granular calcium salts were deposited in proximal tubular epithelia showing necrosis. In the other organs and tissues, there was erosion in the abomasal mucosa.

Bacteria in nasal and pulmonary lesions were gram-negative and pleomorphic bacilli ranging from small rods to filaments or gram-positive coccobacilli in the sections with Gram stain. Numerous \textit{F. necrophorum} biotype A and \textit{A. pyogenes} antigens were detected corresponding to gram-negative bacilli and gram-positive coccobacilli in the nasal and pulmonary lesions. Infiltrated neutrophils and macrophages in the lesions had antigens in their cytoplasms (Figs. 2C and 2D). Neither of the antigens were found in the renal lesions. None of the other antigens were detected in any sections.

In bovine digital arthritis and osteomyelitis with concurrent infection of \textit{F. necrophorum} and \textit{A. pyogenes}, the former bacterium induced a necrotic reaction and the latter a purulent one [7]. \textit{F. necrophorum} hemagglutinin and lipopolysaccharide (LPS) may be responsible for the formation of thrombi in the microcirculation caused by infection [3, 18], and may participate in the initial lesions of the inflammatory process [6]. Rhinitis in the present case was characterized by mucosal coagulative necrosis, multiple thrombosis and fibrinopurulent exudation. Impairment of oxygen transport by the thrombosis and lowered oxygen tension and redox potential following the growth of \textit{A. pyogenes}, a facultative anaerobic bacterium, may have contributed to the establishment of an anaerobic microenvironment enabling \textit{F. necrophorum}, an obligate anaerobic bacterium, to colonise the nasal mucosa. The thrombosis likely played an important role in the development of the nasal lesions in the present case, although the exact cause of the thrombosis is unclear, but possibly may have been due to \textit{F. necrophorum} hemagglutinin or LPS.

Aspiration of nasal exudate in rhinitis can lead to bronchopneumonia [2]. The necrotic bronchopneumonia in the present case was attributed to the aspiration of pieces of infectious material from the nasal mucosa into the lung, since \textit{F. necrophorum} and \textit{A. pyogenes} antigens were detected in the pulmonary lesions.

The factors related to the present renal infarct remain unclear, since none of the examined antigens were found in the renal lesions.

In calf diphtheria associated with \textit{F. necrophorum} infection as the chief pathogen, predisposing factors are required for tissue invasion [10, 18]. They include mucosal injuries caused by viruses, allergens and other bacteria [10, 18]. No antigens other than \textit{F. necrophorum} and \textit{A. pyogenes} were detected in any examined tissues from the present case. A nonspecific stress situation of immediate postparturition in the aged cow, when suffering from the disease, may have facilitated the infection and colonization of bacteria in the nasal mucosa and pulmonary lobes.

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