Pathological Studies on Mucormycosis of the Forestomach and Abomasum in Ruminants: A Report on Six Cases Complicated with Candidiasis or Pulmonary Aspergillosis

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Abstract. Mucormycosis of the forestomach and the abomasum was found in two beef bullocks, three cows and a ram. Clinical examination revealed various symptoms due to anomaly of the alimentary canal in three of the bovine cases antemortem. The others were postparturient downer and acute necrotic mastitis cow respectively. Postmortem examination disclosed three stages of lesion: an acute early stage of lesions of marked circulatory disturbance with necrotic suppurative changes, a subacute middle stage of severe necrosis with suppurative changes, and a chronic late stage of granulomatous lesions. Vascular affinity of fungal hyphae was very common in all the lesions, except those of the chronic late stage. Mucormycosis was associated with disseminated miliary nodular Aspergillus pneumonia in one of the bovine cases and with candidiasis in the ram. Discussion was made on the importance of the histopathological examination in mycotic diseases. Rumen acidosis due to excess feeding with grains seemed to be a main predisposing factor in most of the cases, although no concurrent lesions detected were negligible.

Mucormycosis is caused by infection with the Genera Absidia, Mucor and Rhizopus of Family Mucoraceae of the Order Phycocoeetes. It is known to be divided into two types of disease. One type is surface and the other, deep systemic affection. A number of papers were published on mucormycosis of the alimentary canal in cattle [7, 10, 18, 29, 34, 35, 37, 39], sheep [26, 38], swine [11, 18], dogs [12, 33], cats [18, 36], laboratory animals [31], monkeys [9, 15], and human beings [3, 16, 23, 27, 28]. Reports were made on candidiasis of the organs in calves [22, 41], piglets [1, 11, 20, 32, 42], adult swine [17, 19], cats [36], and human beings [24]. Infection of the upper digestive canal with Candida seems to cause a high fatality rate in piglets [1, 32, 42]. Pulmonary aspergillosis was also mentioned in cattle [5, 8, 13, 14, 25, 48] and lambs [2].

Feeding with excess grains as well as frequent administration with such drugs as antibiotics and steroid hormones, may be enough to induce fungal diseases in animals. The present authors examined 5 bovine cases and one ram case of mucormycosis of the alimentary canal. This disease was associated with pulmonary aspergillosis in one
of the bovine cases and with candidiasis of the skin and the forestomach in the ram case.

Materials and Methods

All the animals examined had been brought from farms in Morioka City and its suburbs, except one, No. 5, used for routine pathological examination. Autopsy was performed when they died or were killed by bleeding at the Department of Veterinary Pathology, School of Veterinary Medicine, Iwate University. Bullock No. 5 was slaughtered at Sakata Abattoir in Yamagata Prefecture.

Detailed observation was conducted at autopsy. Materials were taken from organs and tissues of the whole body, and fixed in 10% neutral formalin solution. Paraffin sections were made and stained mostly with Hematoxylin and eosin (H-E). If necessary, some serial sections were subjected to PAS staining, Grocott’s application to fungi of Gomori’s methenamine-silver nitrate technic, and Gram staining for histological observation.

Results

1. Clinical history and autopsy findings

Case No. 1 (Pr. 674): A seven-year old Holstein-Friesian cow suffered from a sudden onset of anorexia and colic. Four days later, she was laparotomized under a tentative diagnosis of intestinal invagination. During her surgical incision, she died due to excessive hemorrhage on November 27, 1969. There were no records of treatment with antibiotics or steroid hormones.

At necropsy, dirty turbid lesions were found on the mucosa of the abomasum. They were irregularly projected from the mucosal surface, reddish purple in color, and as large as red beans up to 5 cm in diameter. Their central part was grayish in color and touched hard. The uterus contained 8-month-old twin fetuses.

Case No. 2 (Pr. 968): A six-year-old Holstein-Friesian cow was a postparturient downer. She received medical treatment without antibiotics for 3 days, but died on January 23, 1974.

Autopsy revealed indurations varying in size from soybean to thumb-tip on the mucosa of the dorsal sac of the rumen and the folia mucosa of the omasum. They were reddish or grayish brown in color. Besides, right pericoxitis with necrosis of surrounding muscles, suppurative bronchopneumonia, and necrotic endometritis were observed.

Case No. 3 (Pr. 887): A sixteen-month-old Japanese Black beef bullock suffered from persistent mucous diarrhea blackish in color for 10 days. No medical treatment was performed. It died on September 13, 1972 due to advancing anorexia and depression.

Autopsy disclosed ulcers of rice-grain size or linear type 2–3 cm long on the esophageal mucosa. Some lesions were projected from the mucosal surface of the rumen. They were from thumb-tip size to 5 cm in diameter with the denuded mucosa yellowish green in color. Caseous materials were contained in the deep portion of the cut surface of the lesions. Whitish nodules varying in size from pin-point to millet grain were scattered in the parenchyma of the lungs. Some of the bronchioli contained a grayish white turbid substance with the mucosa reddish in color. Besides, the urinary bladder ruptured due to necrotic hemorrhagic cystitis and necrotic serofibrinous pericititis as a result of extravasation of urine.

Case No. 4 (Pr. 767): A fifteen-year-old Holstein-Friesian cow suffered from acute necrotic mastitis. After a week of medical treatment with antibiotics, she was killed by bleeding on October 19, 1970 due to bad prognosis.

Autopsy disclosed many linear ulcerative lesions on the top of mucosal wrinkles of the abomasum and several ulcers of soybean size yellowish brown in color on the mucosa.
of the pyloric region. Diffuse necrotic mastitis, interstitial nephritis, and calcium deposition on the endocardium were also detected.

Case No. 5 (E 1944): A fourteen-month-old Japanese Black beef bullock suffered from bladder calculus and recovered by medical treatment. Afterwards, it showed symptoms of impaction, bloating, and atony of the rumen repeatedly for 10 days. During the course of disease, no antibiotics were administered. It was slaughtered at Sakata Abattoir on September 25, 1973.

The wall of the forestomach was thickened to be 1.5 cm in thickness in almost all the portions. The folia of the omasum reached 7–8 mm in thickness. The mucosa of the forestomach showed necrosis with ulcers and was elastic and hard in touch. Proliferation of translucent granular tissue with multiple miliary abscesses was obvious on the cut surface of the gastric wall.

Case No. 6 (Pr. 1048): A three-year-old Corriedale ram showed a sudden loss of weight and malnutrition. Two weeks later, she exhibited difficulty to stand and killed by bleeding on February 7, 1975. She did not receive any medical treatment during the course of disease.

At autopsy, her wool was easily depilated by fingers in the shoulder and back area. A number of grayish brown or yellowish caseous lesions up to 20 cm in diameter were detected on the mucosa of the forestomach (Fig. 1). The peripheral mucosa of each lesion was reddish in color and hard in touch. The mucosa was desquamative and ulcerative. Besides, hundreds of nodes of the nodular worm varying in size from rice grain to little-finger tip were found on the intestinal wall, and a few of them in the mesenteric lymph nodes and the surface of the liver.

2. Histological findings

Case No. 1: Marked hyperemia and hemorrhage and desquamation of epithelial cells were found on the mucosa of the abomasum (Fig. 2). Many infiltrating round cells, neutrophils, and eosinophils and collapsed cell elements were detected in the tunica propria and muscularis mucosa. Irregularly branching, nonseptate hyphae 4–8 μm in width (Fig. 3) and fungal thrombi (Fig. 4) were commonly observed. The vascular wall was necrotic. The hyphae stained slightly with hematoxylin and were PAS-positive. Histological diagnosis was hemorrhagic suppurative necrotic mucormycosis of the abomasum which appeared to be in the acute early stage.

No obvious lesions were detected in any other organ or tissue, except marked circulatory disturbances.

Case Nos. 2 and 3: Similar lesions were present in the rumen and omasum of case No. 2 and the rumen of case No. 3. They were coagulation necrosis extending from the mucosa to the muscularis of these organs (Fig. 5). Edematous and fibrinous exudate was predominant and accompanied with diffuse infiltration and focal accumulation of neutrophils. Dilation of blood vessels with necrotic walls and thrombi was commonly observed. Numerous hyphae were scattered in the lesions and invaded blood vessels. Necrosis and ulcer were also detected on the esophageal mucosa. No fungal hyphae were demonstrated in the lesion of No. 3. Focal fat necrosis with fungal hyphae was recognized in the adipose tissue of the rumen serosa in No. 3. These hyphae were 4–8 μm in width and morphologically similar to those seen in No. 1. Histological diagnosis was necrosis-predominating mucormycosis of the forestomach which was in the subacute middle stage.

In bullock No. 3, disseminated miliary
lesions were scattered in the lungs which were hyperemic. They consisted of several to dozens of alveoli with neutrophils and collapsed cell debris. The alveolar wall was difficult to distinguish and occasionally remained as a shadow. A radial arrangement of hyphae was exhibited in lesions stained by Grocott's method (Fig. 6). Although it was rather difficult to demonstrate hyphae in H-E stained sections, it was possible to recognize an intracellular substance slightly basophilic in these sections. Septate hyphae were 2–4 μm in width and weakly PAS-positive. Necrosis with cellular infiltration was noticed in the bronchiolar wall and debris with hyphae were in the lumen. Fungal invasion was demonstrated in a branch of the pulmonary artery with thrombi and in a necrotic bronchial cartilage. Many of the hyphae were enlarged in width in these lesions. In general, the hyphae showed less affinity to blood vessels than those of Mucoraceae and occasionally proliferated through the blood vessels. Histologically, the lesion was diagnosed as disseminated necrotic supplicative miliary Aspergillus pneumonia.

Additionally, histological examination revealed bronchopneumonia with diphtheric tracheitis, necrotic supplicative endometritis, pericarditis, diffuse catarrhal mastitis, intestinal catarrh, parasitic cholangitis, chronic interstitial nephritis in No. 2, and chronic necrotic cystitis, urethritis with fibrous peritonitis, chronic interstitial nephritis, and lymph sinus catarrh in No. 3.

Case No. 4: A number of hyphae were observed in the superficial portion of the ulcer base of the pylorus. Multiple abscesses and granulation tissue with a few hyphae were recognized around the ulcerative lesion. Neutrophilic cell infiltration was predominant. The hyphae detected were morphologically similar to those obtained from case No. 1. Histological diagnosis was abscess-forming granulomatous mucormycotic gastritis which was considered to be in the chronic late stage.

Besides, histological examination revealed pulmonary edema, necrotic diffuse mastitis, interstitial nephritis with neutrophilic cell infiltration, neutrophilic cell infiltration in the adrenal medulla and cortex, calcium salt deposition in endocardium and myocardium, and lymph sinus catarrh.

Case No. 5: Necrosis and desquamation of the mucous membrane and granulomatous lesions of nodular structure were observed over an area from the propria to the muscularis in almost every portion of the forestomach. These nodular lesions contained cellular debris and neutrophils with eosinophilic PAS-positive hyphae (Fig. 7). These hyphae still possessed the characteristics of Mucoraceae. They were 4–8 μm or more in width, nonseptate, and branched irregularly. Proliferation of epithelioid cells and Langhans' giant cells was observed. Acidophilic hyphae were occasionally found in the cytoplasm of the giant cell (Fig. 8). Some of the hyphae formed Gram-negative clubs projecting from the surface (Fig. 9). Lymphocytes and plasma cells infiltrated into the surrounding granulation tissue. Fat necrosis was recognized with a few hyphae on the serosa of the rumen (Fig. 10).

A small amount of Gram-positive cocci was accumulated on the ulcerative surface. So-called micro-necrosis was detected in the epithelial layer of the mucosa near the lesion. It consisted of a small focal accumulation of necrotic epithelial cells and neutrophilic cell infiltration. Several transverse sections of calcified nematodes with fibrous capsulation were observed in the subserosa of the rumen. Histological diagnosis was granulomatous mucormycosis of the forestomach which was in the chronic
late stage.
Tubulonephritis without PAS-positive organisms was observed among the other organs examined.
Case No. 6: Focal necrosis and erosion with neutrophilic cell infiltration were recognized on the mucosa of the esophagus where no hyphae were detected. Vacuolar degeneration and desquamation of epithelial cells and a marked neutrophilic cell infiltration were noticed in the mucosa of the rumen. Necrosis with hyperemia and hemorrhage was obvious over an area from mucosa to muscularis. There were many nonseptate hyphae 6–12 μm (8 μm on the average) in width, some of which had invaded blood vessels. Lesions similar to those of the rumen were found in the reticulum and the omasum where necrosis was frequently demonstrated all over the mucosa. Necrosis and ulceration occurred to the mucosa of the abomasum. Neutrophilic cell infiltration and scattered hyphae of Mucoraceae were seen in lesions extending to the muscularis.
Numerous round or oval yeast spores about 2–4 μm in diameter were detected around the wool in keratinous debris on the skin surface. No changes were detected in the deeper part of the epidermis or dermis. Hyperkeratosis of the mucosa with many yeast spores 2–3 μm by 3–4 μm in size and pseudohyphae 2–3 μm in width was observed in PAS-stained sections of the forestomach (Fig. 11). It was characteristic of candidiasis without recognizable intracellular substances.
Histological diagnosis was necrotic abscess-forming mucormycosis of the forestomach and the abomasum which was in the subacute middle stage and accompanied with superficial and desquamative candidiasis of the skin and forestomach.
No detectable changes observed in any other organ, except the presence of numerous parasitic nodes on the intestinal wall and chronic interstitial nephritis.

Discussion
Six cases of mucormycosis were described. They were divided into 3 groups according to the stage of pathologic lesions. In the first group, to which belonged cow No. 1, circulatory disturbances were predominant in lesions of necrotic and supplicative changes. These lesions were generally limited to mucosa and submucosa, and presented characteristics of the acute early stage of disease. The second group were composed of Nos. 2, 3 and 6. In it, coagulation necrosis and infarct were common and accompanied with marked infiltration and accumulation of neutrophils. They were usually found over an area from mucosa to serosa, and represented the subacute middle stage of disease. Nos. 4 and 5 formed the third group. The lesion of this group resembled granulomatous actinomycosis in nature. In it, clubs projected from acidophilic fungal hyphae. The cases of this group were supposed to be in the prolonged chronic late stage of disease.
Schiefer [36] and Shimizu et al. [37] were in the opinion that the necrotic changes mentioned above might have resulted from circulatory disturbances due to the invasion of blood vessels by fungal hyphae. Granulomatous lesions in mucormycosis were described by Gleiser [12], Davis et al. [6], König et al. [18] and Spratling et al. [39] in bovine cases, by Shirley [38] and Morales and Van Kruiningen [26] in ovine cases, and by Gitter and Austwick [11] in swine cases. Among them, those described by König et al. [18] and Spratling et al. [39] were identical with those of bullock No. 5 in the present study.
Serial changes shown in the present study
seem to be important for understanding the pathogenesis of the disease. They may have been induced by host-related factors, such as concurrent lesions, other basic diseases, and intensity of resistance of the host and by parasite-related factors, such as fungal species and rate of infection. The Mucoraceae had little affinity to blood vessels in the granulomatous actinomycosis-like lesions of No. 5. Acidophilic hyphae were fragmentary and frequently phagocytized by giant cells appearing in the lesions. This may indicate an increase in the local resistance of the host, and the appearance of acidophilic hyphae represent a degenerative stage. König et al. [18] reported two types of lesion, chronic granulomatous and acute, and cited Scholer and Richle who were in the opinion that the round and fragmented form of hyphae in the granulomatous lesion might be a half-inhibited one. Further examination may verify this opinion.

It is a well known fact that there are predisposing factors in mycotic infection. They are diabetes [4], leukemia and other chronic exhaustive diseases [16, 23, 36], and long-term treatment with antibiotics or steroid hormones [3, 15, 18, 21, 22] in man and animals [30]. None of the present cases showed any antemortem sign of diabetes. Records indicated that only No. 4 had received unknown doses of antibiotics against mastitis. Various concurrent lesions detected, in the postmortem examination may have been induced by a reduction in the resistance of the host due to mycotic infection. The reticuloendothelial system had only mild activity in the lymph nodes of the whole body in all the cases, even in cases with concurrent lesions of infective nature. From these findings, it was considered that all the animals might have had no resistance or be in an immunodeficient state from the beginning of the disease.

In ruminants, the chemical inflammatory condition of the forestomach and abomasum due to rumen acidosis results from an excess supply of grain food. It is an important factor for the occurrence of mucormycosis in the digestive canal [7, 35, 39, 40]. Recently, a prevailing method of fattening, as well as a predominant manner of feeding milking cows, consists in an excess supply of concentrates. It is reasonable to consider that the excess amount of grain food may be beyond the physiological capacity of digestion in the animal.

The authors had a chance to carry out a histological examination on 47 experimental fattening cattle slaughtered in 1974–75. Of these cattle, twenty-eight (59.6%) possessed foci of micro-necrosis and neutrophilic cell accumulation in the epithelial layer of the forestomach (unpublished data). These changes were similar in nature to those detected in No. 5. Accordingly, if such feeding with concentrates is continued, mycotic gastritis is anticipated to occur frequently in ruminants.

Recently, Neitzke and Schiefer [29] took an interest in relationship between the occurrence of mycotic gastritis and infectious bovine rhinotracheitis (IBR). Such viral diseases affecting the mucous membrane of the digestive canal as mucosal disease, malignant catarrhal fever, and IBR may also act as predisposing factors of the mycotic infection.

Besides, it should be borne in mind that Mucoraceae produces fat necrosis when it invades adipose tissues, as observed in the present cases, Nos. 3 and 5. PinSENT and Ritchie [34] reported a case of mucormycosis in a cow in which there was an adhesion between the perforated abomasum and the omentum affected with fat necrosis.

Three animals examined in the present study presented clinical symptoms due to
disturbances of the digestive canal. They were killed or died at 4–12 days of illness. Colic symptoms [35] and diarrhea [7, 11] were observed in animals reported previously. These results would be of help in making a clinical diagnosis of mucormycosis.

In ram No. 6 affected with cutaneous and deep candidiasis, the lesions of the forestomach may have been preceded by those of the skin. Spores and pseudohyphae propagated on the hyperkeratotic cornal and epithelial layers of the forestomach presented figures similar to those reported by previous authors [1, 41]. There was no direct relationship between lesions of candidiasis and those of mucormycosis observed in the forestomach. Gitter and Austwick [11] reported swine gastritis with lesions identical with those of the present ram which Candida and Rhizopus had invaded. Neitzke and Schiefer [29] found a calf with mucormycotic gastritis associated with Aspergillus and Candida infection.

Unfortunately, no attempts were made to isolate fungi from the present cases. The occurrence of mucormycotic rumenitis with Aspergillus pneumonia in a bullock and with candidiasis in a ram in the present study, however, is a warning against putting too much confidence on the result of fungal isolation. In other words, it may be possible to make an erroneous diagnosis in the case of complicated infection. Accordingly, in making diagnosis of fungal disease, it is necessary to perform histological examination and, as Schiefer [36] expressed, sections should be stained with some specific stains to identify the causative fungus.

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Explanation of Figures

Fig. 1. Necrotic lesions of mucosa of the forestomach in ram No. 6. R: rumen, RE: reticulum, and O: omasum.

Fig. 2. Many thrombi in mucosa and submucosa of the abomasum in cow No. 1. Hyperemia, hemorrhage and desquamation of epithelial cells are seen. H-E. ×44.

Fig. 3. Nonseptate irregularly branched mucormycotic hyphae in mucosa of the abomasum in cow No. 1. PAS stain. ×440.

Fig. 4. Fungal invasion of blood vessels in submucosa of the abomasum in cow No. 1. PAS stain. ×440.

Fig. 5. Coagulation necrosis and cellular infiltration with thrombi in mucosa through muscularis of the rumen in bullock No. 3. H-E. ×44. Inset shows mucormycotic hyphae in muscularis. PAS stain. ×440.

Fig. 6. Aspergillus nodule in the lung of bullock No. 3. Radial growth and Y-branching of hyphae are presented. Grocott's application of Gomori stain. ×220.

Fig. 7. Granulomatous lesion in the rumen mucosa of bullock No. 5. PAS stain. ×44. Inset shows mucormycotic hyphae in neutrophilic cell debris at the center of a granuloma indicated by a thin arrow. PAS stain. ×440.

Fig. 8. Magnification of the area of Fig. 7 indicated by a thick arrow. Fragmentary short hyphae phagocytized by a giant cell in granuloma. PAS stain. ×440.

Fig. 9. Clubs formed around hyphae sectioned transversely and neutrophils scattered in a rumen granuloma of bullock No. 5. H-E. ×440.

Fig. 10. Fat necrosis in rumen serosa with mucormycotic hyphae in bullock No. 5. PAS stain. ×220.

Fig. 11. Many Candida yeasts and pseudohyphae in epithelial layer of rumen mucosa in ram No. 6. PAS stain. ×440.