Pathological Studies on Systemic Mycoses in Calves

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ABSTRACT. Systemic mycoses were found in 19 (4.7%) of 406 calves less than 6 months old which were autopsied during the past 10 years. Alimentary mycosis occurred in 12 (63.2%) of 19 cases. In alimentary mycosis, mucormycosis showed the highest rate of occurrence (91.7%, 11/12 calves) followed by aspergillosis 41.7% and candidiasis was 9.3%. Mucormycosis and aspergillosis were characterized by local hemorrhagic necroses with hyphal proliferation and thrombi in the mucosa and muscular layers of the forestomach, abomasum, and small intestine. Candidiasis was characterized by hyperkeratosis with pseudohyphae and microconidia in the mucosa of the omasum. Four of 12 calves (33.3%) had mixed infections of the alimentary tract consisting of Mucorales and Aspergillus species. Pulmonary aspergillosis was found in 10 (52.6%) of 19 calves. There were micro-abscesses with hyphal proliferation or asteroid bodies in the lungs. Infections involving both the alimentary tract and respiratory organs were noted in 3 (10.5%) of 19 calves. Disseminated mycosis was found only in one calf. In alimentary mycosis, administration of antibiotics for the treatment of diarrhea and early weaning were thought to be an important predisposing factor.---KEY WORDS: aspergillosis, calves systemic mycosis, candidiasis, mixed infection, mucormycosis.


The fungal infections are divided into superficial or dermatomycoses and deep-seated or systemic mycoses (SM) [5, 22, 33], or superficial, cutaneous, subcutaneous, systemic and miscellaneous infections depending on the affected organs or tissues [31].

SM have been well known due to opportunistic infections [31, 37]. Although there have been many reports about SM in calves [6-13, 15-20, 23, 26, 27, 29, 35, 40], reports concerning the rate of incidence of SM in calves have been limited [9, 13, 16, 18, 29].

This report describes the results of pathologic examinations of SM in calves autopsied during the past 10 years.

MATERIALS AND METHODS

Four hundred and six calves less than 6 months old were autopsied at the Department of Veterinary Pathology, School of Veterinary Medicine, Rakuno Gakuen University, from April, 1975 through March, 1985. Blocks of organs and tissues were fixed in 10% neutral formalin solution, and paraffin sections were cut and stained with hematoxylin and eosin (HE) by routine methods. For detection of hyphae, the periodic acid-Schiff (PAS) reaction and Gomori's methenamine silver (GMS) stain were done.

Nineteen calves of SM were found among the 406 autopsies (Tables 1–3). Fifteen calves were Holstein-Friesian (HF) and 4 were Japanese Black breed. There were fourteen female calves and 5 males. Calves 1–7 were HF male and raised in the same feed-lot. About 70 calves, all HF male were raised in the same stable in the early winter, from November to December. Each 7-day-old calves were introduced from other farms to the feed-lot.

Table 1. Incidence of systemic mycosis in calves less than 6 months old during 10 years

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<td>Autopsy cases</td>
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<td>Mycosis</td>
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The calves were fed artificial milk, hay and pellets of concentrated feed. Purulent nasal discharge was recognized in 80% of calves about 10 days later, and antibiotic therapy was begun for the affected calves. Calves 1, 4 and 6 showed bloody diarrhea and about 10 of the total died after oral administration of antibiotics. Calves 8–19 were sporadic cases kept separately on different farms. Oral administration of antibiotics was given to calves 8, 9, 11, 14 and 18 which showed diarrhea.

For mycological examination, pieces of tissue were soaked in 0.1% mercuric chloride solution for 30 minutes, washed twice with sterilized distilled water, minced, and incubated on malt extract agar with chloromycetin at 37°C for 2 days.

RESULTS

Incidence of SM

Annual and seasonal incidences of SM are shown in Tables 1 and 2. The incidence of SM during the past 10 years was 4.7% (19/406 calves). The distribution of mycotic lesions is shown in Table 3.

Among the 19 calves, 12 alimentary mycoses, 10 pulmonary mycoses, 3 complicated infections of both alimentary and respiratory organs, and 1 disseminated mycosis were recorded.

Thirteen cases of aspergillosis, 11 mucormycosis, 4 mixed infections with Aspergillus and Mucorales, 1 candidiasis, and 1 complicated infection with Aspergillus of the small intestine and Candida in the
esophagus and omasum were found.

Alimentary mycosis (AM)

Incidence and gross findings: Alimentary mycosis was detected in 12 calves examined. Lesions were noted in the rumen (6 calves), reticulum (6 calves), omasum (2 calves), abomasum (3 calves), and intestine (4 calves).

Histopathologically, 5 aspergillosis, 11 mucormycosis, 4 mixed infections with Aspergillus and Mucorales, 1 candidiasis, and 1 complicated infections with Aspergillus and Candida were found in the 12 calves. A single infection of Aspergillus was seen in the small intestine (calf 9) and abomasum (calf 14). Other lesions of aspergillosis were also infected with Mucorales.

The autopsies showed forestomachs that contained muddy contents with hay or fibrous material and caseous milk. The contents also had putrid smell, and were firmly adhered to the mucosal surface of the forestomach in some instances.

The gross lesions were varied in degree. In the minimal lesions, focal hemorrhagic necroses (5×7 mm - 20×30 mm) were found on mucosa of the forestomach and abomasum. In advanced lesions, larger hemorrhagic necroses or ulcers (1×2 cm - 8×9 cm) were noted (Fig. 1). Erosion (5×7 mm) were scattered on the omasal mucosa in candidiasis.

Hemorrhagic necroses (5×7 mm - 20×30 mm) showing a slight bulge and ulcers were seen in the small intestine (Fig. 2).

Caseous focal necroses were found in the walls of the forestomach (calves 5, 6, 8, 12) and small intestine (calves 6, 12). There were no hemorrhagic changes surrounding the necrotic areas.

Histopathological findings

Mucormycosis: Hyphal proliferation with neutrophil infiltration were easily seen in the degenerated epithelial layer and lamina propria of the forestomach. Hemorrhagic necroses with marked thrombi containing the hyphae were found in the area between the lamina propria and submucosa in advanced lesion (Fig. 3).

Hyphae were nonseptate, aberrant, and bulbous in the lesions. The width of the hyphae varied from 4 to 10 μm. Hyphae branched irregularly with vacant cytoplasm. The hyphal walls were thin and visualized by PAS and GMS staining (Fig. 4). Some hyphae branched frequently at right-angles. Many thrombi, and marked proliferation of hyphae in the vascular wall and thrombi were noted. These hyphae showed the characteristics of a member of order Mucorales.

In the small intestine, similar lesions as seen in the forestomach were found. Granulomatous lesions containing the hyphae were observed in the submucosa of the small intestine of calf 17.

Aspergillosis: Hyphae proliferated parallel to the surface of degenerated hyperkeratotic stratum corneum in the forestomach. Occasionally hyphae invaded the epithelium and penetrated vertically into the lamina propria and submucosa. Following the proliferation of the hyphae, necrotic lesions were seen. Marked neutrophil infiltrations were found at the marginal zone of the lesions. Thrombi with hyphae were also found in the lesions. Focal hemorrhagic necrosis with fragmented hyphae were noted in the abomasum and small intestine. Hyphae proliferated vertically into the submucosa (Fig. 5). Some hyphae invaded and penetrated the vascular walls (Fig. 6). The hyphae detected in the lesion had thin parallel walls with septa stained deeply with PAS. Their width ranged from 3.2 to 4.8 μm. They showed Y-shaped dichotomous branching with an angle of about 45°. The hyphae contained a basophilic substance stained with HE and diffusely deep black with GMS stain in the cytoplasm (Figs. 5, 6, 7).

Lesions of mixed infection with Aspergillus and Mucorales: In the lesions of mixed infection, both hyphae of Mucorales and Aspergillus sp. were found (Fig. 7). Hyphae of Mucorales showed more preference to blood vesseles than those of Aspergillus sp.

Candidiasis: Marked parakeratosis, hyperkeratosis and desquamation of epithelial cells were seen on the epithelium of the tonsilar crypts, and the mucosa of the esophagus and omasum in calf 9. Acidophilic microcomidia were found in HE stained sections (Fig. 8). Pseudohyphae were clearly demonstrated by PAS staining. They were diffusely located in the mucosa to the germinal layer (Fig. 9). A few lymphocytes infiltrated into the lamina propria.

Mycotic lesion related to necrobacillosis: There were coagulation necrosis due to necrobacillosis in the forestomach and intestine. Hyphal proliferation of Mucorales and Aspergillus (calf 5), Mucorales (calf 6) or Aspergillus (calf 8) were found in the lesions of necrobacillosis. No cellular reactions were found surrounding the necrotic areas.

Pulmonary mycoses (PM)

Incidence and gross findings: PM were found in 10
Fig. 8. Hyphae of *Candida* sp. in the hyperkeratotic stratum corneum of the omasum. Calf 9. HE. ×180.

Fig. 9. Hyphae of *Candida* sp. showing characteristic budding in the stratum corneum of the omasum. Calf 9. PAS. ×360.

Fig. 10. Characteristic hyphae of *Aspergillus* showed radial proliferation and Y-shaped branching in the lung. GMS and HE. Calf 15. ×360.

Fig. 11. Microabscess and marked neutrophil infiltration in the lung. Asteroid body is seen in the center of the abscess. Calf 1. HE. ×90.

Fig. 12. Asteroid body and neutrophil infiltration in the lung. Calf 19. PAS. ×360.

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Fig. 1. Diffuse and focal hemorrhagic necroses on the mucosa of rumen and reticulum. Calf 14.

Fig. 2. Focal hemorrhagic necrosis on the intestinal mucosa. Calf 6.

Fig. 3. Hemorrhagic necrosis of the rumen. Marked neutrophil infiltration and thrombi are seen in the mucosa and submucosa. Mucormycosis. Calf 10. HE. ×90.

Fig. 4. Hyphae of Mucorales with marked neutrophil infiltration. Rumen. Calf 14. GMS and HE. ×360.

Fig. 5. Hyphal proliferation of *Aspergillus* sp. from the mucosa to submucosa with thrombus in the intestine. Calf 14. GMS and HE. ×90.

Fig. 6. Fungal thrombi in the lamina propria of the intestine. Hyphae of *Aspergillus* penetrate into the vascular wall. Calf 9. GMS and HE. ×180.

Fig. 7. Mixed lesion of aspergillosis and mucormycosis. Hyphae of *Aspergillus* sp. showing Y-shaped branching in the muscular layer of the intestine. Calf 14. GMS and HE. ×360.
of 19 calves (Table 3). All of them were consistent with aspergillosis caused by aerogenous infection.

At autopsy, purulent catarrhal bronchopneumonia, purulent fibrinous bronchopneumonia or pulmonary abscess were seen. Abscesses (1 mm in diameter) containing caseous or sticky pus were scattered in the lung parenchyma.

**Histopathological findings:** Hyphae of *Aspergillus* sp. were found in abscesses. A few hyphae showed the radial proliferation and Y-shaped branching (Fig. 10). Asteroid bodies with acidophilic druse were found in micro-abscesses in 8 of 10 calves (Table 3) (Figs. 11, 12). Five of 8 calves showed slight granulomatous reactions with Langhan's giant cells and epithelioid cells around the necrotic foci. Granulomatous nodules were revealed only in calf 19. Lesions in the bronchus showed a tendency of expansion, and the granulomatous lesions extended to the surrounding alveoli.

One of the calves (calf 14) developed disseminated lesions. Detail of the findings had already published elsewhere [6].

**Mycological findings**

The results of mycological examination are shown in Table 3.

**Underlying diseases and predisposing factors**

Underlying diseases and predisposing factors were as follows; pneumonia (9 calves), diarrhea treated with antibiotics (10 calves), alimentary disturbance due to inadequate early weaning (4 calves), septicemic infections (2 calves), long-term cortico-steroid treatment (1 calf), lymphosarcoma (1 calf), leptospirosis (1 calf), white muscle disease (1 calf), cardiac malformation (1 calf), and weak calf syndrome (1 calf).

**DISCUSSION**

Although, the opportunity of mycotic infection is increasing because of the extensive use of chemotherapeutic and immunosuppressive drugs in human medicine [1, 22, 31], no tendency to increase in occurrence of mycosis was found in calves during the past 10 years surveyed in the present study. The incidence of SM in the present survey was 4.7% (19/406 calves).

The monthly incidence of SM varied with more cases occurring in the late fall and spring.

With AM, Neitzke and Schiefer [29] found 12 (3.8%) mycotic gastritis cases in 308 autopsied calves that were less than 30-days age during a period of one year. Gitter and Austwick [13] found abomasal mucormycosis in 7 (3.4%) of 208 calves autopsied during a period of 3 years. In the present examination, the incidence of AM was 3.0% (12/406 calves). This rate was similar to the incidences described by Neitzke and Schiefer [29] and Gitter and Austwick [13]. Although the abomasum was one of the organs most often affected with AM [13, 29], a high rate of occurrence was recognized in the forestomach in the present examination.

There are many reports on mucormycosis [2, 9, 13, 23, 29, 35] as well as aspergillosis [17, 27, 29] in calves, but in general the incidence of mucormycosis is higher than aspergillosis in the alimentary tract [34]. Neitzke and Schiefer [29] investigated 12 cases of mycotic gastritis of calves and identified all of them as phycomyctes (mucormycosis) with one mixed infection with *Aspergillus* and phycomyctes. These studies were similar to the present result; primary aspergillosis is considered to be uncommon in the alimentary tract [6, 36].

Mucormycosis is the most frequent fungal infection in the alimentary tract especially in the forestomach. On the other hand, aspergillosis is predominant in the respiratory organs.

Candidial infection of the alimentary tract, especially in the forestomach was reported in calves [10, 16, 26, 41], but the incidence of candidiasis was quite low. In the present study, candidiasis was found only in one calf. Detection of hyphae under HE stained section was not easy and only microconidia were demonstrated. By PAS staining pseudohyphae as well as microconidia were strongly positive.

Mixed infection was reported in selected papers [13, 29, 30]. The rate of mixed infection was 21% (4 calves) and was common in the present study.

Disseminated mycosis was reported in calves [8, 9, 17, 20, 26, 35], but the incidence was as low as only 1 calf [6] was found in the present study.

Complicated infections of the alimentary tracts and respiratory organs has been described [29] and considered to be uncommon, which was also a low incidence in the present study.

The histological findings were different between the aspergillosis and mucormycosis in AM. In the acute aspergillosis, cellular reaction for hyphae was weak and necrosis was present around the hyphae. In mucormycosis, neutrophils reacted from the early stage of infection without effective relationship to
the growth of Mucorales. The difference of tissue reaction in both species is considered to be related to the toxicity of aspergillosis [31, 38]. Marked thrombus formation was seen in mucormycosis than in aspergillosis.

All mycotic affection was aspergillosis in the respiratory organs, and most of the hyphae were found in the intrabronchiolar abscess, which were considered to be the secondary infection. The lesions of aspergillosis in the lungs were similar to the cases reported [12, 15]. Eggert and Romberg [12] stated that pulmonary aspergillosis resulted from the inhalation of spores from moldy bedding.

Austwick [3] detected 36 lesions of aspergillosis (73%) in the investigation of 49 pairs of lungs of dairy cows and found asteroid bodies in 30 lesions (61%). He suspected that there were many cases of chronic infection continued from their calf age. Marcato et al. [24] also reported granuloma of mycosis in the lungs and detected 59 (12.8%) of 461 cases of aspergillosis. Asteroid bodies were found in 8 of 10 calves, and granulomatous reaction was found in 5 of 8 calves in our case. Symmers [39] said that aspergillus body contains gammaglobulin supporting the view of eosinophilic deposits are a morphological manifestation of an antigen-antibody reaction. Matsui et al. [25] reported pulmonary aspergillosis in apparently healthy young rabbits younger than five weeks old. Asteroid bodies were found microscopically in the central area of the lesions with aggregations of heterophils. Additionally, projections that were associated with the cytoplasm of the surrounding heterophils were found using electron microscopy. Matsui et al. [25] determined that the formation of asteroid bodies occurred in the rabbits following primary infection with Aspergillus sp. and was not considered to be an immunological response.

Mucormycosis, aspergillosis and candidiasis were well known as the opportunistic infections [5, 31, 38]. There have been many discussions about the pathogenesis of AM. Neitzke and Schiefer [29] suggested that mycotic gastritis may have started on the basis of an erosive rhinitis due to infectious bovine rhinotracheitis virus infection. Cordes et al. [8] and Hogben [18] said that SM in very young calves might have resulted from an intraterine infection. In calves, rough feed may cause the damage to the mucosa of the stomach and lead to mycotic infections [13]. Antibiotic treatment for diarrhea was considered to be a predisposing factor [4, 10, 21, 26, 28, 29, 41]. In our cases (calves 1–7), they were weaned early and fed hay and pellets. Most calves of the present AM contained muddy and badly fermented material in the forestomach, which suggested disturbances of the organ. Gitter and Austwick [14] discussed the pathogenesis of mucormycosis and moniliasis in a litter of suckling pigs, and stated caseous contents in the stomach cause the hyperemia and erosion of the mucosa, and secondary mycotic infection would occur. Sanford [32] emphasized the importance of administration of antibiotics as a cause of mucormycosis in suckling pigs. Functional disturbance of alimentary tract due to early weaning and administration of antibiotics for diarrhea were thought to be important predisposing factors in the present study.

There was an inconsistency between the results of the mycological and histological examinations in this report. Histopathological examination should be emphasized for a correct diagnosis of mycotic infections.

REFERENCES

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