Disseminated Aspergillosis with Lesions in the Central Nervous System in a Calf

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(Rceived 12 January 1987/Accepted 13 October 1987)

ABSTRACT. A 10-day-old calf was affected with disseminated aspergillosis involving the central nervous system. The primary lesion was thought to be bronchioli. The metastatic lesions were found in the kidney, heart, lymph node and ischiadic nerve. Alimentary aspergillosis and mucormycosis were observed independently. Weak constituting resulting from premature delivery, dislocation of the right hip joint and administration of antibiotics and dexamethasone were considered as predisposing factors for the mycotic infection.—KEY WORDS: aspergillosis calf, central nervous system.

Bovine disseminated aspergillosis (DA) occurs rather infrequently and has been reported in only a few cases in cattle [4, 5] and calves [6, 7]. Among them, the central nervous system (CNS) was affected only in one case [6].

Four hundred and six calves less than 6 months old were autopsied at the Department of Veterinary Pathology, Rakuno Gakuen University, from April, 1975 through March, 1985. Out of 19 calves affected with systemic (deep seated) mycosis, only one case was DA with CNS lesions and independent alimentary aspergillosis and mucormycosis. This report describes pathologic findings of the calf.

MATERIALS AND METHODS

A male Japanese Black calf was born on April 2, 1983 by premature delivery. He was a small physique (15 kg weight) at birth and unable to stand or suckcolostrum without aid. As a black diarrhea was noted on the 2nd day after birth, he was treated with intravenous and oral liquid transfusions. On the 3rd day, marked anemia (Ht: 23%) was observed. Mycilline (5 ml) was administered on the 2nd, 3rd, 4th, 5th and 8th days and dexamethasone (3 mg) on the 3rd day. Oral liquid transfusion was continued until the 9th day. He died on the 11th day.

After routine autopsy, materials were fixed in 10% neutral formalin solution. Parafin sections were made by the routine procedure and stained with hematoxylin and eosin (HE). For the detection of mycelial elements, periodic acid-Schiff (PAS) reaction and Gomori's methenamin silver (GMS) stain were adopted on selected sections.

RESULTS

At autopsy purulent catarrhal broncho-pneumonia with abscesses sized from sesame- to rice-grain was observed in the cranial and medial lobes of the lung. Yellowish red focal necrosis was scattered in both ventricular and septal myocardium.
Yellowish infarcts sized rice-grain were found in the cortex of both kidneys.

In the CNS there was an increase of bloody and turbid cerebrospinal fluid,

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<th>Aspergillosis</th>
<th>Mucormycosis</th>
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<td>Kidney</td>
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<td>Ischiadic nerve</td>
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\textsuperscript{a} Central nervous system

marked hyperemia and scattered submeningeal hemorrhages sized broad-bean to rice-grain in both hemispheres. The olfactory bulbial ventricles, lateral ventricles and aqueous duct of the midbrain were filled with coagulated blood. On examination of sliced section after fixation, there were submeningeal hemorrhages mainly located in the cerebral sulcus and deeper part of the white matter.

Sporadic hemorrhagic necrosis of varying sizes from the tip of the little-finger to the palm, covered with a yellowish pseudomembrane, was found on the rumen mucosa. Focal hemorrhagic necrosis of about red-bean size was noted on the mucosa of the reticulum, omasum and ostium omasoabomasicum. Soy-bean-sized focal hemorrhagic necrosis was scattered on the abomasal mucosa. A protruded center of necrosis was reddish black and encircled by

![Fig. 1. Necrosis in a bronchiolus and the surrounding alveoli. Necrotic vasculitis due to an invasion of hyphae is noticed (arrows). HE. $\times 36$.](image1)

![Fig. 2. A radial proliferation of hyphae in the center of a bronchiolar necrosis. Hyphae shows septa, dichotomous branching and vesicular swelling. GMS and HE. $\times 180$.](image2)
fresh reddish hemorrhage. In the colon there were many hemorrhagic ulcers of varying sizes from red-bean to thumb-tip with fibrinous pseudomembrane.

Dislocation of the right hip joint, and general anemia due to hemorrhages were observed in the muscle around the right hip joint and on the serosa of the pelvic cavity.

Microscopically, aspergillosis lesions were seen in the kidney, heart, lung, rumen, omasum, abomasum, colon, bronchial lymph node, tonsil, ischiadic nerve and CNS (Table 1).

In the lung neutrophilic infiltration and cellular debris were observed in the lumen of the bronchioli. Hyphae and a few asteroid bodies were detected in the cellular debris. The hyphae invaded into the proprial and muscular layer of the bronchioli, accompanied with neutrophilic infiltration and tissue necrosis. There was large necrosis extending from the bronchiolus to the surrounding alveoli (Fig. 1). Hyphae have proliferated radially from the center of the necrotic lesions (Fig. 2) and invaded the arteriolar wall causing necrotic thromboarteriolitis (Figs. 1 and 3).

In the bronchial lymph node focal necrosis extending from the capsule to the medulla, and proliferations of hyphae and hyphal thrombi in the capsule were observed. The follicles were small with scarce cellular components.

In the heart focal hemorrhagic necrosis with hyphal proliferation and thromboangitis, and neutrophilic reaction was scattered around the myocardium. The necrotic lesions were clearly demarcated from the surroundings. Hyphal intravascular proliferation was evident (Fig. 4). Hyphae invading the myocardium from the blood vessels were orientated parallel to the myocardial fibers and showed a radial or finger-like appearance (Fig. 5).

In the kidney wedge-shaped infarcts were found in the cortex. There were necrotic thromboangitis with perivascular neutrophilic infiltration and invasion of Aspergillus hyphae.

In the ischiadic nerve hyphae proliferated in both perineurium and endoneurium with neutrophilic infiltration.

In the CNS mycotic lesions were recognized on the parietal and temporal lobes of the right hemisphere, frontal and temporal lobes in the left hemisphere, left diencephalon, mesencephalon and cerebellum. In the cerebrum there was suppurative hemorrhagic fibrinous meningitis with hyphal thromboangitis. Wedge-shaped necrosis was observed from the meninges to the superficial layer of the white matter involving the sulcus (Figs. 6 and 7). There were prolonged hyphae running in capillaries, associated with perivascular neutrophilic infiltration. Occasionally, the hyphae invaded the parenchyma through the blood vessels, accompanied by neutrophilic reaction (Figs. 7 and 8). There were discoloration, spongiosis, atrophy and ischemic changes in the nerve cells. Focal demyelination with hyperemia, hemorrhages and neutrophilic...
cells. In the granular layer there was evident hyphal thrombosis and perivascular neutrophilic infiltration. Focal hemorrhagic necrosis was noted in the mesencephalon and fibrinous hemorrhagic meningitis in the medulla oblongata.

Focal hemorrhagic necrosis with neutrophilic infiltration was present from the mucosa to the serosa in the rumen and omasum. Hyphal thrombovasculitis was detected in the muscular layer of the rumen. Large necrosis with hyphal invasion, peripheral vascularization, and lymphocytic and histiocytic reaction was observed from the mucosa to the muscular layer of the abomasum. Hemorrhagic necrosis with hyphal invasion was found from the mucosa to the muscular layer of the colon.

In the tonsil necrosis of the follicles with asteroid bodies was observed. A cellular depletion was noted in the lymphoid tissue.

The hyphae detected in the lesions had a thin parallel wall with septa stained deeply with PAS. Their width ranged from 3.2 to 4.8 μm, with an average of 4 μm. They showed Y-shaped dichotomous branching
with an angle of about 45°, and were arranged and orientated in the same direction giving brush-like, finger-like, or radial appearance. The hyphae contained basophilic substance with HE stain in the cytoplasm and were stained diffusely deep black with GMS stain. Some hyphae showed vesicular swelling and a pattern of dichotomous short branching.

Besides the lesions due to aspergillus, mucormycosis was detected in the rumen, reticulum, omasum, small intestine and spleen (Table 1). Mucormycotic lesions neighboring the aspergillosis lesions were observed from the mucosa to the serosa of the rumen and omasum, in which Mucorales hyphae invaded the blood vessels and formed venous thrombi. Focal necrosis was noted in the reticulum from the mucosa to submucosa. Diffuse hemorrhagic necrosis was observed from the mucosa to serosa of the small intestine. Mucorales hyphae proliferated in the necrotic foci.

In the spleen there was hemorrhagic fibrinous necrotic capsulitis accompanied with neutrophilic infiltration, vascularization and hyphal proliferation, and hemorrhagic infarcts due to hyphal necrotic thromboangiitis. The hyphae invaded the parenchyma as well as the trabecula from the capsule. Follicles were undeveloped.

Hyphae detected in the lesions were nonseptate, aberrant or bulbous, and showed a hazard and right-angled branching. Their width ranged from 4 to 6 μm, with an average of 6 μm. Vesicular swellings of the hyphae were often noted in an average width of 8 μm, with a maximum of 12 μm.

**DISCUSSION**

Hyphae in the present case were considered to be a member of the *Aspergillus* sp. from the morphological characteristics, especially Y-shaped dichotomous branching and thin parallel wall with septa [1, 10]. Aspergillosis is referred to as a variety of disease conditions caused by several species of *Aspergillus*, and known as an opportunistic infection [1, 10, 11]. The rate of the opportunistic infection is proportional to the
use of antibiotics, cytotoxins, immunosuppressive drugs, steroids, and other disruptive procedures to the normal flora that results in lowering host resistance [10]. It is well known that disseminated aspergillosis could result from one or combinations of the following three factors: (1) lowered resistance due to debilitating disease or drugs, (2) local point of entry for the fungus (barrier break) and (3) disruption of normal flora and inflammatory response by antibiotics and/or steroids [10].

Administration of antibiotics for the treatment of diarrhea seems to be an important factor in mycotic infection of the alimentary tract in calves [4, 8]. Sheridan [12] noted that 87% of the systemic bovine mycosis followed the use of antibiotics for enteritis. In the present case, the calf was born by premature delivery, revealed a dislocation of the hip joint and colostrum-suckling insufficiency, and received dexamethasone and antibiotics treatment. He also showed undeveloped follicles and less cellular components in the lymphoid organs. Pulmonary and disseminated aspergillosis and alimentary mucormycosis in the present case might be induced by the lowered host resistance to infection.

According to our experience, the rate of disseminated mycosis was 0.3% of calves autopsied in our Department, and 5.3% of ones affected with systemic mycosis. When systemic mycosis occurred, calves died by acute course with only the primary lesion, rarely revealing the dissemination.

The main route of infection of bovine DA is known to be the alimentary tract and disseminated lesions were noted in the liver [4–6]. No mycotic lesion was found in the liver of this case. Mycotic invasion to the arterioli was noted in necrotic lesions of the bronchioli which were considered as the primary lesion. The aspergillosis lesions in the heart, kidney, ischiadic nerve and CNS were supposed to be disseminated lesions.

Lesions observed in the CNS were resulted from hyphal thromboangitis and essentially diagnosed as thrombo-embolic meningo-encephalitis with parenchymal necrosis and demyelination. These lesions were similar to those of what has been described for cerebral mucormycosis [2].

The hyphae which were nonseptate, aberrant or bulbous and showed a haphazard and right-angled branching were considered to be a member of the order Mucorales [2]. The mucormycotic lesions in the spleen were considered to have been extended from the ruminal serosa.

Double infection of aspergillosis and mucormycosis has been reported in selected papers [8, 9], and histopathological investigation on the mycosis was emphasized [3, 9].

ACKNOWLEDGEMENTS. The authors wish to thank Professor K. Ohshima, of the Department of Veterinary Pathology, Faculty of Agriculture, Iwate University, for his valuable advice and review of this manuscript and Dr. K. M. Gabbar, of the Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, for his valuable advice of this manuscript.

REFERENCES

DISSEMINATED ASPERGILLOSIS IN A CALF


要約
中枢神経系に病変のみられた仔牛の播種性アスペルギルス症の1例：千早 豊・松川 清・吉澤幸夫・八田嘉明・岡田洋之・石井幸二（酪農学園大学家畜病理学教室，石狩地区農業共済組合江別家畜診療所）——過去10年間における当教室での仔牛の剖検例406例中に19例の深在性真菌症を認めた。そのうちアスペルギルス症の1例の中枢神経，腎臓，心臓，気管支リンパ節および末梢神経に播種性病変が認められ，原発巣は肺と考えられた。さらに消化器系アスペルギルス症およびムコール症を伴っていた。早産による虚弱体質，出生時の股関節脱臼，抗生物質およびデキサメサゾンの授与による感染への抵抗性の低下が真菌感染の前駆要因と考えられた。