A HISTOPATHOLOGICAL REPORT ON A CASE OF HISTOPLASMOSIS IN A HEIFER WITH FALLOT'S TETRALOGY

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Histoplasmosis is a disease caused by *Histoplasma capsulatum* among human beings and animals^{2,3,26,27}). The occurrence of the disease is enzootic^{15,18}) and has been reported from various parts of the world³). The fungus is a sort of saprophytes and distributed in the soil of the world. Occasionally, it propagates in animal tissues after an infection. Accordingly, no features of communicable diseases can be seen¹⁶). The fungus is diphasic and grows easily in a tube, in which it shows the mycelium phase as seen in natural soil. On the contrary, in animal tissues, the yeast phase is a common feature of the propagation²).

DE MONBREUN⁸) reported a canine case in 1939. That is the first report in domestic animals. Since then, many investigations have been reported in the central eastern district of U.S.A.^{15,16,17,34}) Occurrence of the disease was observed in Canada⁹) and Central³³) and South America¹⁴). The fungus has been isolated from various kinds of domestic^{2,19,20,23,24,26,28}) and wild animals^{2,20}), in which proliferation of the reticular tissues and endothelial system has been recognized. Many yeast-phase fungi are demonstrable in the cytoplasm of the proliferated cells. The disease is very common in dogs^{6,7,8,12,26,30,31,34}). Foci of granulomatous cells are usually formed without necrotic foci in the lesion. Giant-cell formation demonstrated in the lesion of another kind of fungal diseases is rarely observed in histoplasmosis. The histopathology of the disease is characteristic. The presence of demonstrable yeast-phase organisms 2-5 μ in diameter in the cytoplasm on histological sections is useful for a histological diagnosis of the disease⁴).

According to MENGES et al.¹⁹), ADLER¹) reported a generalized histoplasmosis in a range bull in Hawaii. MENGES and KINTNER¹⁷) investigated a bovine case in Missouri that showed a severe circulatory disturbance due to traumatic reticulitis, and found cell nests of endothelial leukocytes with *H. capsulatum* in their cytoplasm at periportal areas in the liver. MENGES et al.¹⁹) demonstrated the organisms in the lungs and bronchial lymph node of a calf from Kentucky by means of the fluorescent antibody technic and cultural method. They examined the lymph node histologically and found granuloma tissues with a calcified necrotic center but not *H. capsulatum* in the lesions.

A considerable number of bovine cases were reported to have shown a positive reaction in the histoplasmin skin test^{10,15,19,28}). FURCOLOW and RUHE¹⁰) examined with histoplasmin and pointed out that 10.3% of positive cases had been found among cattle over 8 years old, and that 5.1% among the examined cases including younger ages of Kansas cattle. MENGES and KINTNER¹⁷) reported that 45.5% of positive cows were over 8 years old in Missouri. SALAW et al.²⁹) produced experimental infection of the disease by

the route of air passage and noticed temporary clinical symptoms. They reported that it was difficult to find out any lesion at autopsy and that negative results had been obtained in isolation of the organisms from the tissues. A review of literature revealed that the organisms seemed to affect the bovine host easily but form lesions only occasionally. The present authors had a chance to investigate a bovine case of histoplasmosis with heart defects of FALLOT's tetralogy^{22,32}).

METHODS

For the pathological examination, tissues and organs were obtained from the whole body after a minute observation at autopsy and fixed in neutral 10% formalin solution. Paraffin sections were made by the routine method and stained with hematoxylin and eosin for general examination. Many of them were also treated with PAS stain, luxol fast blue and PAS double stain, thionin stain, and ZIEHL-NEELSEN stain.

CASE REPORT

History

The experimental animal used was a Holstein-Friesian heifer which was born on a farm in Iwate Prefecture. Tachypnea and marked cyanosis of visible mucous membranes had been recognized after a short time of exercise since she was 3 months old. On August 11, 1966, she was hospitalized in the Iwate University Veterinary Clinic.

Examination revealed an undernourished 12-month-old heifer weighing 190 kg and suffering from anorexia and severe general cyanosis. Her body temperature was 39.2°C, pulse rate 96, respiratory rate 42. The respiratory rate sharply increased even after a slight movement. There was marked pulsation on the cervical veins. Systolic murmur was heard over a wide area. No abnormality was detected in the stomach movement or intestinal peristalsis. Hematological examination disclosed a finding of concentrated blood with a red blood cell count of 21,420,000, a white blood cell count of 8,100, and a differential leukocyte count consisting of 1.5% non-segmented neutrophils, 35.5% segmented neutrophils, 27.5% eosinophils, 28.5% lymphocytes, and 7.0% monocytes. The hematocrit value was 95.0%, the hemoglobin value 29.5 gm/dl, the viscosity 6.75, the specific gravity 1.102. She was kept under observation and examined clinico-physiologically for 125 days until death occurred^{22,32}).

Autopsy

Heart defects indicating the evidence of FALLOT's tetralogy were confirmed. They were (1) interventricular septal defect of thumb size in the upper portion, (2) pulmonic stenosis of little-finger size and transposition of the artery to the left, (3) dextroposition and riding of the dilated aorta, and (4) hypertrophy of the right ventricle resembling the left ventricle. Additionally, (5) marked passive hyperemia of the whole body, the dark reddish mucous membrane of the digestive canal, and the purple reddish lungs with hard consistency were observed. Moreover, (6) many caseous necrotic nodules which were well marginate and of millet to rice-grain size were detected on and in the kidneys, spleen, heart muscle, and brain. The Inhalt of each lesion was poorly encapsulated and hard to squeeze out. Besides, (7) a soybean-sized defect of mucous membrane was found on the back of the tongue.

Histopathology

Caseous necrotic lesions were detected in the spleen, kidneys, heart, and brain. They were characteristic and measured up to 3 mm in diameter. A caseous substance

surrounded by cellular debris was located at the center of each lesion. The necrotic area was surrounded by proliferated histiocytes with broad cytoplasm (Fig. 1). Scanty fibrous tissue and moderate cell infiltration of lymphocytes, plasma cells, and neutrophils were also observed. Vascular proliferation and disappearance of myelin were noticed in the histiocytic layer in brain lesions, some of which had no necrotic area. Many PAS-positive, yeast-like organisms were found in the cytoplasm of the proliferated histiocytes. They were stained poorly with hematoxylin and $1\sim5\mu$, mostly $2\sim4\mu$, in diameter (Figs. 2~4). Many yeast-like organisms $1\sim3\mu$ in diameter were recognized in the crevices of necrotic debris in lesions of the kidney. They were PAS-positive and showed metachromasia with thionin stain (Fig. 5). No ZIEHL-NEELSEN-positive organisms were detected in or around the lesions.

Liver: There were marked hyperemia and atrophy of cell cords with pyknotic nuclei. The endothelial cells of the sinusoid were enlarged and contained hemosiderin pigments. Occasionally, there were small foci composed of naked nuclei, as well as nuclear debris, in the sinusoid.

Spleen: No hyperplastic changes occurred to the malpighian corpuscles. Cell-poor pulp with red blood cells and numerous granules of hemosiderin pigment were recognized. Several caseous necrotic lesions gathered together in or along the splenic sinuses, forming large lesions about 2 mm in longer diameter.

Kidneys: There was marked hyperemia especially in the medulla. Caseous necrotic lesions about 3 mm in diameter were present in the cortex. Some of them were solitary and others gathered together to form a lesion of soybean size. The structures of renal tubules still remained in the area of cell proliferation. The capsule of BOWMAN was thickened with fibrous proliferation. Cellular and proteinic casts were observed in the renal tubules located near the lesions. Many of the epithelial cells were swollen up, presenting degenerative desquamation.

Lungs: Marked hyperemia, dilated capillaries containing blood, and marked hemorrhages into the alveolar spaces were observed. Organisms stained slightly with hematoxylin were detected on the alveolar wall, presenting various phases of Histoplasma, including mycelium and yeast phase, as well as transitional conversion cells. They were PAS-positive and occasionally $3\sim4\mu \times 70\sim80\mu$ in size. Most of them were less than 20μ in length. Branches projecting into the alveolar spaces were seen in some of the cells (Figs. 6 and 7).

Heart: Blood vessels dilated with blood. Intermuscular tissue was edematous. Caseous necrotic lesions were in muscular tissue and subendocardial or subepicardial tissue. Cellular debris had accumulated in intermuscular connective tissue. PAS-positive yeast-like organisms were in degenerated cells.

Tongue: An ulcerous lesion was observed in the mucous membrane and the propria layer. There were necrotic tissue debris and marked neutrophilic cell infiltration. Large histiocytic cells were proliferated in the lesion. Many PAS-positive yeast-like organisms were present in the cytoplasm (Figs. 8 and 9).

Rumen: Small necrotic lesions about 300μ in diameter were observed in the epithelial layer. They contained cellular debris with tiny filamentous thread-like and coccus-like PAS-positive substances.

Intestine: There were marked hyperemia, desquamation of epithelial cells, and cell infiltration in the propria layer.

Skeletal muscles: Focal areas showed marked hyperemia and dilatation of blood capillaries. Tiny PAS-positive dots were seen in enlarged endothelial cells. Disappearance of the striae, atrophy and fragmentation of muscle fibers were observed.

Lymph nodes: Proliferation of sinus reticular cells and infiltration of neutrophilic cells were observed in many nodes. Marked hyperemia and hemorrhage were recognized in renal and iliac nodes. Cobble-stone-like arrangement of epithelioid reticulum cells with PAS-positive yeast-like organisms was observed in the nodes.

Bone marrow: Collagenous appearance of adipose tissue and hematopoiesis were distinctly recognized.

Central nervous system: Transverse sections were prepared from the frontal, lateral, parietal, and occipital lobes, corpus striatum, interbrain, midbrain, pons, medulla oblongata, and cerebellum for examination. One to three lesions were detected in every section. The lesions were about 3 mm or less in diameter. They were located in both grey and white matter and occasionally under the meninges.

Other organs: No obvious changes were observed.

DISCUSSION

Lesions characteristic of histoplasmosis were found in visceral organs of the heifer examined. They were the proliferation of histiocytes around necrotic foci and the presence of PAS-positive organisms of yeast phase $2\sim 4\mu$ in diameter in these cells. BADER and ROHDE⁴⁾ presented a key for the histological diagnosis of the tissue form of pathogenic fungal species. According to them and other previous authors^{2,6,8,12,17,25,26,30)}, the organism described above was identical with *Histoplasma capsulatum*, although no bacteriological cultural examination was carried out in the present study. Reticulum cells, or cells of the reticuloendothelial system (RES), have been regarded as host cells of *Histoplasma*. Since the histogenesis of the brain lesion was clarified in the present study, the histiocyte in the wide sense of the word would be a better term for the host cell of *Histoplasma*.

The mycelium-phase, yeast-phase, and transitional conversion cells¹¹⁾ detected in the lungs were proved to be organisms identical with one another from a morphological point of view; that is, on the basis of size, shape, and tinctorial characteristics. MOORE²¹⁾ reported two human cases in which the mycelium-phase *Histoplasma* had been detected from the lung. According to him, HALEY obtained similar results from an experiment with mice. The mycelium phase of the fungus is a saprophytic phase growing easily in a tube. The fungus is sensitive to CO_2 , and its mycelium phase fungus is transformed easily into a yeast phase when cultivated in a tube containing added CO_2 ⁵⁾. The mycelium phase may be allowed to grow in the lung due to abundant oxygen supply.

Digestive tract^{23,25)} and respiratory passage³⁰⁾ have been reported to be entrance routes of the etiological organism. In the present case, which showed no focal lesions in the lung but ulcerous lesions on the mucous membrane of the tongue, the digestive tract might be suggested as the route. It was difficult to identify the organisms detected from the necrotic lesions on the rumen mucosa as *H. capsulatum*.

Establishment of infection with the saprophytic fungus in the present case would depend on the conditions of the host that had been suffering from illness for a long time and showed marked circulatory disturbances of FALLOT's tetralogy. There are some similarities in host conditions between the present case and MENGES and KINTNER's case¹⁷⁾ that was associated with traumatic reticulitis. It is impossible to estimate the time of infection on the basis of the lesions examined or the data obtained by clinical examination.

Findings of histoplasmosis in Japan are very scarce. There were only reports on two human cases^{13,35)}. Both of the cases concern residents in foreign countries. No reports have been published on domestic animals so far as the authors know. Accord-

ingly, deniable factors are too many from an epidemiological point of view. However, the results of histopathological examination and unusual conditions of the host were useful for the diagnosis of histoplasmosis in the present case.

SUMMARY

Histopathological examination was made on a case of generalized disseminated histoplasmosis with FALLOT's tetralogy.

Typical lesions were recognized in the spleen, kidneys, heart, brain, and tongue. They had a rather distinct margin and consisted of a caseous necrotic central area and a surrounding area of histiocyte proliferation with cell infiltration. Many yeast-phase organisms, which were possibly *Histoplasma capsulatum* from a histopathological point of view, were detected in the cytoplasm of the proliferated cells and in crevices full of necrotic debris in some of the lesions. They were spherical in shape, PAS-positive, and 1~5 μ , mostly 2~4 μ , in diameter. Mycelium-phase organisms were detected from the alveolar wall in the lungs.

The present case would be the first one reported in Japan among domestic animals and the 4th bovine case with lesions in the world.

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FALLOT 四徴を示した心奇形牛にみられた ヒストプラズマ病について

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臨床的に FALLOT 四徴を示し、かつ解剖学的に心奇形を確認した牛における、全身性散種性ヒストプラズマ病について、主として病理組織学的研究を行なった。

本病において普通にみられる病巣が、脾、腎、心、脳および舌粘膜に認められた。これらの病巣は、中心部は乾酪化物質および細胞性頽廃物をいれ、これを取り囲んで組織球性細胞の増殖がみられた。ここには、病巣によってはリンパ球、形質細胞、好中球などの細胞浸潤を伴い、毛細血管の

新生像を示すものもあった。増殖した組織球胞体内には、病理組織学的観点から *Histoplasma capsulatum* と考えられる菌体を多数認めた。これらはヘマトキシリンに淡染、PAS-強陽性、チオニンに異染性を示す直径 1~5 μ 、大部分は 2~4 μ の酵母相を示した。また、一部病巣の中心部頽廃物の裂隙には、同様の菌体を多数いれるものもみられた。肺においては、強い充出血と、肺胞壁における菌糸相、酵母相および移行期の菌体が多数認められた。

家畜における本病は、わが国では、この例以前に知られていたものはない。また、牛において病巣が確認されたものとしては、この例は世界で第4例と目され、極めてまれな症例と考えられる。

これらのことから、検索例の病変論に併せて、本病の疫学、宿主条件などについても、若干の論議を行なった。

EXPLANATION OF PLATES

PLATE I

- Fig. 1. Caseous necrotic lesion in the occipital lobe of the brain. Caseous substance with cellular debris is surrounded by a layer of histiocytic proliferation (arrow) with vascular proliferation. HE stain, $\times 40$.
- Fig. 2. A high-power magnification of the arrow portion in Fig. 1. Cobble-stone-like arrangement of proliferated histiocytes with slightly stained organisms in their cytoplasm. HE stain, $\times 400$.
- Fig. 3. Section successive to Fig. 2. PAS-positive yeast-phase organisms in histiocytic cytoplasm. PAS stain, $\times 400$.
- Fig. 4. A layer of histiocytic proliferation around a necrotic lesion in the kidney. Numerous PAS-positive yeast-phase organisms (arrows) in proliferated cells. PAS stain, $\times 1,000$.
- Fig. 5. Caseous necrotic lesion in the kidney. Numerous PAS-positive yeast-phase organisms $1-5\ \mu$ in diameter in a crevice filled with debris. PAS stain, $\times 400$.

PLATE II

- Fig. 6. Hyperemia and hemorrhage in alveolar spaces of the lung. PAS stain, $\times 100$.
- Fig. 7. Many PAS-positive mycelium- and yeast-phase organisms and transitional conversion cells on alveolar walls of the lung. PAS stain, $\times 1,000$.
- Fig. 8. Ulcerous lesion on the mucosa of the tongue. HE stain, $\times 40$.
- Fig. 9. A high-power magnification of the arrow portion in Fig. 8. Many proliferated histiocytes with PAS-positive yeast-phase organisms in their cytoplasm. PAS stain, $\times 400$.