Pulmonary Mycosis associated with Selenium-deficient Cardiomyopathy in Sika Deer

Kazuki YOSHIOKA¹⁾, Hideaki UEKI¹⁾, Shuiqin WANG²⁾, Yangxian LI²⁾, Zhiping XIA²⁾,
Hiroyasu YOSHIKAWA¹⁾, Toshifumi OYAMADA¹⁾ and Takashi YOSHIKAWA¹⁾

1)Department of Veterinary Pathology, Faculty of Veterinary Medicine and Animal Sciences, Kitasato University,
35-1 Higashi 23 Banchou, Towada City, Aomori 034-8628, Japan

2)Department of Veterinary Medicine, Changchung University of Agriculture and Animal Sciences,
175 Xian Road, Changchung, 130062 Jilin Province China

(1999.11.9 received, 1999.12.24 accepted)

シカのセレンウム欠乏性心筋症にみられた肺真菌症

吉岡 一機¹⁾, 植木 秀彰¹⁾, 王 水 琴²⁾, 李 養 賢²⁾,
夏 志 平²⁾, 吉川 博康¹⁾, 小山田 敏文¹⁾, 吉 川 堯¹⁾

1)北里大学獣医畜産学部獣医病理学講座 〒034-8628 青森県十和田市東二十三番町 35-1

2)長春農牧大学獣医学院 〒130062 中国吉林長春市西安大路 175

ABSTRACT. Pulmonary mycosis of four domestic sika deer (*Cervus nippon* Temminck and *Cervus elaphus*) aged 1 to 2 weeks suffering from selenium deficiency (enzootic cardiomyopathy) were examined histopathologically, histochemically and by electron microscopy. From the morphological features of mycosis there were diagnosed aspergillosis in three cases and mucormycosis in one case. The pathologic reaction within lesions of aspergillus were dominated by granulomatous type with neutrophils, macrophages and giant cells, and mucor lesions were remarkable vasculitis with fungal thrombi. It is suggested that the atrophy of lymph follicles and decrease number of T lymphocytes in lymphatic organs is closely correlated with selenium deficiency and immunological disorders.

Key Words : cardiomyopathy, mycosis, selenium deficiency, sika deer.

Jpn. J. Zoo Wildl. Med. 5(1) : 87-92, 2000

INTRODUCTION

A number of papers were published on aspergillosis of the respiratory organs in cattle [1-3], sheep [4] and horses [5-7], but there are few reports of mycotic infection in deer [8-10]. Munro et al. [9] and Jensen et al. [8] are cited that the causal agents of mycotic infection in deer (*Cervus elaphus* and *Dama dama*) are stress and intake of mycotic contaminated food. It is also known that majority

of mycosis is infected with opportunistically, accompanying basic diseases such as chronic inflammation, leukemia and malignant tumors, or administration of antibiotics and steroid hormones [3, 5, 8, 11-13]. This report describes a pathological study of pulmonary mycosis, associated with selenium deficiency, in farmed sika deer (*Cervus nippon* Temminck and *Cervus elaphus*) in the north-east of China (Jilin province).

Table 1 Materials

CaseNo	Age(Week)	Sex	Species	Main pathological findings
1	1	♂	<i>Cervus nippon</i> Temminck	Cardiomyopathy Scatter of caseous nodules in lung Atrophy of spleen
2	1	♀	<i>Cervus nippon</i> Temminck	Cardiomyopathy Scatter of caseous nodules in lung Atrophy of spleen
3	2	♂	<i>Cervus elaphus</i>	Cardiomyopathy Scatter of caseous nodules in lung Atrophy of spleen
4	2	♂	<i>Cervus nippon</i> Temminck	Cardiomyopathy Congestion and pulmonary edema Atrophy of spleen and thymus

MATERIALS AND METHODS

The animals are 1 to 2 weeks old four sika deer affected with enzootic cardiomyopathy, as reported previously [14] (Table 1).

Light Microscopy

Lung specimens were fixed in 10% buffered formalin without delay, dehydrated in a graded alcohol series, cleared in xylol, and embedded in paraffin wax. Tissue sections of 4 to 6 μm thickness were stained by haematoxylin and eosin (HE), periodic acid-Schiff (PAS), Grocott's methenamine silver method (GMS) and Gridley staining for fungi.

Electron Microscopy

Small blocks of lung were prefixed rapidly in 0.05 M glutaraldehyde, postfixed in 1% osmic acid and embedded in epoxy resin (Epok 812) by standard procedures. The embedded specimens were sectioned at 1 μm thickness and stained in toluidine blue for light microscopical observation. Ultrathin sections were double stained in uranyl acetate and lead citrate staining and observed under a transmission electron microscope (Hitachi H-7000).

RESULTS

Gross Findings

At autopsy, markedly congestion and purulent

catarrhal bronchopneumonia, and in three cases (Case Nos. 1-3), many nodules (1 mm in diameter) containing caseous or sticky pus were scattered in the lung parenchyma (Fig. 1).

Light Microscopy

Macroscopic nodules in the lung of Case Nos. 1-3 were characterized by well-demarcated areas of necrosis disposed in stellate fashion. Architectural arrangement of nodules consisted of central areas of necrosis surrounded by zones of cellular infiltration, haemorrhage, and edema. The cellular infiltrates were neutrophils, macrophages and giant cells. Hyphae elements frequently extended beyond the central necrosis into these inflammatory zones.

Fig. 1 Gross findings, scatter of many nodules containing caseous or sticky pus in lung.

Pulmonary Mycosis of Sika Deer

Fig. 2

(A) *Aspergillus* hyphae, arrangement of a radial pattern. GMS. $\times 560$.

(B) *Mucor* hyphae, vasculitis with fungal thrombi. PAS. $\times 280$.

Within the central necrosis, numerous PAS and GMS positive hyphae were arranged in a radial pattern (Fig. 2 A). The fungal organism was characterized by 3 to 4 μm wide septated hyphae with parallel walls and regular dichotomous branching (Fig. 2 A). These hyphae often proliferated in the terminal bronchi or bronchiole cavities, spreading through the bronchial membrane, smooth muscle and elastic fiber to the surrounding the lung tissue. Occasionally, hyphae arranged in a radial pattern were detected in some blood vessels growing from the wall to the vascular lumen. The reaction of the lymphocytes to granulomatous lesions was either lacking or slight. In another case (Case No. 4), on the other hand, the lung lesions in all lobus were congestion and edema, while there was a marked infiltration of neutrophils. At the same time these suppurative changes, vasculitis with fungal thrombi was a frequent observed. A penetrate of the

hyphae from the vascular lumen, which caused the vascular lesion, was also observed (Fig. 2 B). The hyphae lacked a septum and their thickness and branching was irregular (Fig. 2 B). They were eosinophilic and reacted positively to PAS and GMS staining but negatively to Gridley staining. No granulomatous reaction to these hyphae was seen. Other points of particular note were atrophy of the follicles in spleen and lymph nodes, and a marked reduction in number of lymphocytes in T-dependent areas (Fig. 3).

Electron Microscopy

Ultrastructural examination of the hyphae obtained from pulmonary lesions of Case No. 2. The hyphae showed remarkable thickenings (0.3 to 1.3 μm) of the outermost hyphal wall layer. This thickening was composed of poorly differentiated anastomosing microfilaments of reticulate electron dense substance. The innermost hyphae wall layer

DISCUSSION

Fig. 3 Atrophy of the follicles in spleen.
HE. $\times 100$.

was electron transparent and ranged from 0.3 to 0.6 μm in thickness. Intracytoplasmic organelles were relatively well defined (Fig. 4).

Four cases of pulmonary mycosis of sika deer were described. They were divided into 2 groups according to the type of hyphae. Three cases of aspergillus (Case Nos. 1-3), which had septa, were of almost regular thickness, and showed a characteristic dichotomous branching, and one case of mucor (Case No. 4), which had no septa, irregular thickness, and characteristic proliferation within blood vessels along with thrombus [2, 8, 9]. The granulomas are a characteristic lesion of aspergillosis [2, 8], but the spread of inflammation causes from terminal bronchi or bronchiole inflammation which then extent the surrounding tissues.

Ordinary bacterial inflammation processes in the terminal bronchi, bronchiole and alveolar duct, and different from aspergillus pneumonia [15]. Gowing and Hamlin [12] suggested that the necrosis and vascular damage seen in Hodgkin's disease and leukemia infected with *Aspergillus fumigatus* is due to

Fig. 4 The outermost hyphal wall of aspergillus composed of poorly differentiated anastomosing microfilaments (arrow) of reticulate electron dense substance. $\times 20000$.

Pulmonary Mycosis of Sika Deer

the diffusible toxic metabolites produced by hyphae. Kothary et al. [16] reported that the elastase produced from aspergillus play a role in its invasion, and experiments by Yokota et al. [17] on the effect of Asp-hemolysin produced by aspergillus on mice showed necrosis in cardiac muscles and liver cells, suggesting that Asp-hemolysin might play a role in the development of the mycotic infection.

The vasculitis and destruction of elastic fibers seen in alveolar wall, and microfibrils observed in ultrastructure of hyphae suggest the participate of a toxic substance produced by aspergillus. Sukroongreung and Thakerngpol [18] state that the microfibrillar material is a true extracellular component. Various factors are given for the emergence of mycotic infection [11, 13]. The important factors are immunological condition and the existence of any basic disease.

All the cases we examined were less than 2 weeks old, and they suffered from myocardial disease. Myocardial disease in deer, which occurs in the northeastern part of China, is similar in nature to a human disease in the same region called Keshan disease, and its correlation with selenium deficiency is strongly doubted from its epidemiological background and pathological characteristics [14, 19-21]. Taylor [22] suggests that, since messenger RNA with T lymphocyte-related genes (CD 4, CD 8 and HLA-DRp 33) has the open reading frame that codes for selenoprotein, selenium is essential for the proliferation of lymphocytes, and a deficiency in selenium leads to a reduction in lymphocyte and impaired immunofunction.

Kukreja and Khan [23] reported atrophy and T lymphocyte reduction in the spleen and thymus in experimental selenium-deficient rats. The atrophy and particularly the reduction in T-dependent area of the lymphatic organs seen in all the cases examined suggest a correlation with immuno-depression and selenium deficiency.

要 約

セレンウム欠乏性心筋症に罹患した1~2週齢の梅花鹿(*Cervus nippon* Temminck)3例および馬鹿(*Cervus elaphus*)1例,計4例の肺真菌症について病理形態学的に検討した。菌糸の形態から *Aspergillus* 症(3例), *Mucor* 症(1例)と診断され,前者は肉芽腫性病巣,後者は血栓を伴う脈管炎が特徴的であった。真菌感染の成立に対し,検索例で共通的に認められたリンパ系器官の萎縮,特にTリンパ球の減少およびセレンウム欠乏と免疫機序との関連が示唆された。

キーワード: 心筋症, 真菌症, セレンウム欠乏, 鹿

REFERENCES

1. Austwick PKC. 1962. The presence of *Aspergillus fumigatus* in the lungs of dairy cows. *Lab Invest* 11: 1065-1072.
2. Chihaya Y, Furusawa Y, Okada H, Matsukawa K, Matsui Y. 1991. Pathological studies on systemic mycoses in calves. *J Vet Med Sci* 53: 1051-1058.
3. Chihaya Y, Okada H, Matsukawa K, Matsui Y. 1992. Disseminated mycoses in cattle. A study on nine autopsy cases. *J Vet Med Sci* 54: 485-491.
4. Perez V, Corpa JM, Garcia Marin JF, Aduriz JJ, Jensen HE. 1998. Mammary and systemic aspergillosis in dairy sheep. *Vet Pathol* 35: 235-240.
5. Carrasco L, Tarradas MC, Gomez-Villamandos JC, Luque I, Arenas A, Mendez A. 1997. Equine pulmonary mycosis due to *Aspergillus niger* and *Rhizopus stolonifer*. *J Comp Pathol* 117: 191-199.
6. Hattel AL, Drake TR, Anderholm BJ, McAllister ES. 1991. Pulmonary aspergillosis associated with acute enteritis in a horse. *J Am Vet Med Assoc* 199: 589-590.
7. Slocombe RF, Slauson DO. 1988. Invasive pulmonary aspergillosis of horses: An association with acute enteritis. *Vet Pathol* 25: 277-281.
8. Jensen HE, Jorgensen JB, Schonheyder H. 1989. Pulmonary mycosis in farmed deer: Allergic zygomycosis and invasive aspergillosis. *J Med Vet Mycol* 27: 329-334.
9. Munro R, Hunter AR, Bonniwell M, Corrigall W. 1984. Systemic mycosis in Scottish red deer (*Cervus elaphus*). *J Comp Pathol* 95: 281-289.
10. Sato Y, Matsuura S. 1998. Gastric mucormycosis in a sika deer (*Cervus nippon*) associated with proliferation of *Clostridium perfringens*. *J Vet Med Sci* 60: 981-983.
11. Conesa D, Rello J, Valles J, Mariscal D, Ferreres JC. 1995. Invasive aspergillosis: A life-threatening complication of short-term steroid treatment. *Ann Pharmacother* 29: 1235-1237.

Kazuki YOSHIOKA et al.

12. Gowing VFC, Hamlin IME. 1960. Tissue reactions of aspergillus in cases of Hodgkin's disease and leukemia. *J Clin Pathol* 13: 396-413.
13. Meyer RD, Rosen P, Armstrong D. 1972. Phycomycosis complicating leukemia and lymphoma. *Ann Intern Med* 77: 871-879.
14. Yoshioka K, Ueki H, Kurotaki T, Oyamada T, Yoshikawa H, Yoshikawa T, Wang S, Li Y, Xia Z. 2000. Myocardial lesions of enzootic cardiomyopathy in sika deer (*Cervus nippon* Temminck). *J Jpn Vet Med Assoc* 53: 101-104.
15. Kobzik L, Schoen FJ. 1994. The Lung. In *Robbins pathologic basis of disease*, 5th Edit. (Cotran RS, Robbins SL, Kumar V eds.), pp. 673-734. W. B. Saunders Company, Philadelphia.
16. Kothary MH, Chase Jr.T, Macmillan JD. 1984. Correlation of elastase production by some strains of *Aspergillus fumigatus* with ability to cause pulmonary invasive aspergillosis in mice. *Infect Immun* 43: 320-325.
17. Yokota K, Shimada H, Kamaguchi A, Sakaguchi O. 1977. Studies on the toxin of *Aspergillus fumigatus*, VII. Purification and some properties of hemolytic toxin (Asp-hemolysin) from culture filtrates and mycelia. *Microbiol Immunol* 21: 11-22.
18. Sukroongreung S, Thakerngpol K. 1985. Abnormal form of *Aspergillus terreus* isolated from mycotic abscesses. *Mycopathologia* 91: 47-52.
19. Bo-qi G. 1983. Pathology of Keshan disease: A comprehensive review. *Chin Med J* 96: 251-261.
20. Ge K, Xue A, Bai J, Wang S. 1983. Keshan disease an endemic cardiomyopathy in China. *Virchows Arch A Pathol Anat Histopathol* 401: 1-15.
21. Li G, Wang F, Kang D, Li C. 1985. Keshan disease: An endemic cardiomyopathy in China. *Hum Pathol* 16: 602-609.
22. Taylar EW. 1995. Selenium and cellular immunity: Evidence that selenoproteins may be encoded in the +1 reading frame overlapping the human CD 4, CD 8, and HLA-DR genes. *Biol Trace Elem Res* 49: 85-95.
23. Kukreja, R, Khan A. 1997. Effect of experimental selenium deficiency and its supplementation on lymphoid organs. *Indian J Exp Biol* 35: 952-956.