Marble Spleen Disease in Pheasants in Korea

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ABSTRACT. Two pheasants maintained in outdoor closed pen died within several days after having a history of depression. On necropsy, the spleens from both pheasants were enlarged about 3 times of their normal size and appeared mottled in color varying white to red. Histopathologically, there were diffuse severe follicular necrosis in the spleen and congestion and edema in the lung. Intranuclear basophilic inclusion bodies, which are strongly positive to group II avian adenovirus with immunohistochemistry, were noted in the spleen.

KEY WORDS: group II avian adenovirus, marble spleen disease, pheasant.

Marble spleen disease (MSD) was first described in 1966 in Italy [9] and numerous sporadic or endemic outbreaks have been reported worldwide since then [2, 3, 6, 11]. MSD has been known to occur mainly in 3 to 8 months old pheasants that are maintained in confined pens [2, 10]. MSD virus is classified into the group II avian adenovirus and is closely related to viruses that cause hemorrhagic enteritis (HE) of turkeys and splenomegaly of chicken [5, 11]. MSD virus is serologically indistinguishable from that of HE virus. The virus is icosahedral, non-enveloped, and 70 to 90 nm in diameter. Congestion and edema of the lung and splenomegaly with mottled appearance are characteristic gross lesions of MSD [11]. Furthermore, the presence of necrosis or depletion of lymphoid follicles, reticuloendothelial cell hyperplasia and intranuclear inclusion bodies together with splenomegaly are regarded pathognomonic for MSD [11]. In this paper, we describe a case of group II avian adenovirus infection in two pheasants; this is the first report of the case in Asia.

Two male pheasants of 3 or 4 months old which were kept in outdoor pens enclosed by wire-mesh were found dead and submitted to the Department of Avian Medicine, College of Veterinary Medicine, Seoul National University for postmortem examination. Both pheasants were brought from a farm that recently had experienced about 12% mortality among 50,000 flock of pheasants. Young pheasants ranging from 3 to 6 months of age were affected mainly and most of them were usually found dead without any notable clinical histories except that some of them appeared depressed or showed respiratory difficulty before death.

On necropsy, the significant gross findings were limited to the lung and spleen in both cases. The lungs were generally congested and edematous. The spleens were markedly enlarged about 3 times of their normal size and were diffusely mottled in white. The spleens and lungs were fixed in 10% neutral buffered formalin, routinely processed and stained with hematoxylin and eosin (H&E) for histopathologic examination. Immunohistochemical identification of group II avian adenovirus was performed on replicate paraffin sections of the spleen as previously described [8]. The standard avidin-biotin-peroxidase complex (ABC) method was used according to the manufacturer’s protocol (Vetastain kit, Vector Laboratories, Burlingame, CA, U.S.A.) to demonstrate antigen, using 3,3-diaminobenzidine as the chromogen. A mixture of unlabeled monoclonal antibodies directed against HE was used as primary antibody. These monoclonal antibodies are specific to group II avian adenovirus and proven to be not cross-reactive with group I avian adenovirus [12]. The monoclonal antibody pool was diluted 1:2 in phosphate-buffered saline and sections were treated with the primary antibody for 45 min at 37°C. To improve antibody reactivity with antigen, the slides were treated with 0.05% protease (Sigma, St. Louis, MO, U.S.A.) for 10 min at 37°C. Spleen from an pheasant experimentally infected with MSD virus and spleen from an uninoculated pheasant served as positive and negative control, respectively. Portions of the lung, liver, heart blood, and spleen were collected aseptically and routine aerobic and anaerobic bacterial cultures were performed. For virology, spleen and lung homogenates were inoculated into either chorio-allantoic membrane of 10-day-old specific pathogen free embryonated chicken eggs or chicken embryo fibroblast and liver cell cultures.

Microscopically, the lungs were mildly congested. In the spleen, the lymphoid follicles were diffusely necrotic or depleted and fibrinoid material was accumulated in and around the necrotic lymphoid follicles (Fig. 1). Mild to moderate reticuloendothelial cell hyperplasia was also noted. Numerous, intranuclear basophilic to amphophilic inclusion bodies causing margination of the nuclear chromatin were noted in the reticuloendothelial cells (Fig. 2). Immunohistochemically, the inclusion bodies in the spleen were stained strongly positive for group II avian adenovirus...
antigen (Fig. 3). Bacteriology and virology failed to isolate any pathogen responsible for the splenic and pulmonary lesions. Even though virology was not successful in isolating the adenovirus from the lung and spleen, based on the gross and histopathologic findings as well as immunohistochemistry, it could diagnose that those pheasants were infected by MSD of pheasants. Virus isolation is neither a handy procedure for a routine work nor always successful in MSD infection. In countries with endemic MSD, an agar gel precipitin assay is routinely performed on either splenic tissues or sera to diagnose the disease.

Since MSD outbreaks frequently recur annually, carefully controlled vaccination against MSD is highly recommended when confirmed the diagnosis. At present, application of tissue culture-propagated MSD virus vaccine through drinking water has controlled the outbreak of disease successfully so far [1]. There is no known effective treatment regimen for MSD. The disease has been recognized only in confined pheasants and is believed to spread through fecal contamination [10]. Therefore, decreasing the population density during an outbreak might help limiting the spread of MSD [7]. It is also possible that infected pheasants may be treated by injection of convalescent antiserum obtained from healthy pheasants at farm [4]. Like avian circo virus, group II avian adenovirus has immunosuppressive effect and therefore treatment for secondary bacterial infection must be considered also [11].

Commercial pheasant farming comprises a large portion in Korean poultry industry for meat production. Understanding epidemic pattern of the disease and establishment of a disease database is necessary for better management of this species in Korea. Pheasants have not been vaccinated against MSD virus in Korea prior to this outbreak. No other MSD-infected pheasants have been found in that farm since the first outbreak. This report represents the first recognized outbreak of MSD virus infection in Korean pheasants, and poses a significant health and economic threat to the poultry industry. Institution of a controlled MSD-vaccination program, increased biosecurity practices throughout the pheasant industry to control spread of this disease, and continued surveillance of pheasant flocks for additional outbreak of MSD are recommended to help control this disease.

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REFERENCES