MARBLE SPLEEN DISEASE OF RING-NECKED PHEASANTS

A Continuing Education Program Prepared by

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INTRODUCTION

Marble spleen disease (MSD) is a contagious disease of confinement-raised pheasants. It has been a significant cause of mortality in many areas of the United States, Canada, and Europe during the last 30 years. Marble spleen disease is caused by a type II avian adenovirus. The type II adenoviruses include marble spleen disease virus of pheasants, hemorrhagic enteritis virus of turkeys, and splenomegaly virus of chickens, which are morphologically and serologically indistinguishable. However, they are serologically unrelated to type I adenoviruses.

MSD affects pheasants between 2 and 8 months of age. The virus is transmitted laterally, most likely through ingestion of material contaminated by feces. Pheasants are frequently found dead without any previous clinical signs; mortality varies from 5% to 15% of the flock. The course of a natural outbreak is generally 10 to 14 days.

At necropsy, the spleen and lungs are the only organs with gross alterations. The spleen is markedly enlarged and mottled. The lungs are heavy and edematous. Microscopically, the spleen has marked reticuloendothelial cell hyperplasia and characteristic intranuclear inclusions.

There is no specific treatment for MSD infected birds. Antibiotics to prevent secondary bacterial infections, reduction of bird density, and strict sanitation to prevent further virus spread may help to limit mortality. The first effective vaccines against MSD were developed from splenic extracts from birds infected with either MSD or hemorrhagic enteritis. More

recently, several commercial vaccines based on cell-culture-propagated virus have become available.

Since ring-necked pheasants are an important captive-reared gamebird species, this program was prepared to summarize current knowledge on MSD to assist avian diagnosticians with recognition and diagnosis of the disease.

SLIDE SET

1. A mature ring-necked pheasant cock in breeding plumage. Young adult pheasants are generally affected by MSD between 2 and 8 months of age. Respiratory distress is often the only clinical sign preceding peracute death. Mortality rate in naturally occurring outbreaks ranges from 5 to 15%, and the course of the disease varies from 10 to 14 days. Marble spleen disease has been reported only in intensive captive-rearing operations. The disease has not been detected in wild birds.

2. Four spleens from ring-necked pheasants. The 3 on the right are from birds infected with MSD virus and are 2 to 3 times as large as the spleen from a non-infected bird (on the left).

3. Four spleens from ring-necked pheasants infected with MSD virus. Notice the mottling or marbling of the spleens, which accounts for the name of the disease. The pallor and mottling extend all the way through the spleen on section.

4. The lung is another organ grossly affected in MSD. The lungs are heavy and edematous. In natural outbreaks of MSD, birds generally die due to pulmonary edema. The pathogenesis of the pulmonary edema is unknown and has not been reproduced experimentally.

5. Histologic appearance of a section of normal pheasant spleen (H&E, X16). Note the thin layers of pale-staining reticuloendothelial cells surrounding splenic vessels and the prominent basophilic lymphoid follicles.

6. Histologic appearance of a section of spleen from a pheasant infected with MSD virus (H&E, X16). The overall pallor is due to marked reticuloendothelial cell hyperplasia and necrosis and depletion of lymphoid follicles. Reticuloendothelial cell hyperplasia is a nonspecific response and is also seen in many septicemic conditions in response to antigenic stimulation.

7. Higher magnification of a section of spleen from a MSD virus-infected bird (H&E, X100). The lymphoid follicle in the center has lymphoid depletion and patchy lymphoid necrosis characterized by the presence of pyknotic debris. Surrounding the lymphoid follicle is pale, eosinophilic, amorphous material (fibrinoid material). Moderate numbers of heterophils are scattered through the splenic parenchyma.

8. At very high magnification (H&E, X200), a section of spleen from a MSD virus-infected bird contains numerous intranuclear inclusions within reticuloendothelial cells. Inclusions vary from eosinophilic to pale basophilic. Affected nuclei are markedly enlarged, with marginated chromatin. The combination of gross splenomegaly and mottling, with typical microscopic intranuclear inclusions, is nearly pathognomonic for MSD in ring-necked pheasants. There are also numerous heterophils within the splenic parenchyma. This is a variable histologic feature in cases of MSD and is seen in the early stage of infection.

9. Recently, immunohistochemical staining techniques using specific antibodies against MSD viral antigens have been developed. This section of spleen from a MSD virus-infected bird was stained using an indirect streptavidin-biotin immunoperoxidase method (X200). The virus-containing reticuloendothelial cells stain bright red, while the remainder of the cells are basophilic due to the hematoxylin counterstain. This method may be useful when inclusions are rare and difficult to find using standard hematoxylin and eosin staining procedures.

10. As previously demonstrated, the lung is the second organ affected by MSD virus infection. In this low magnification section of lung from a MSD virus-infected bird (H&E, X25), there is diffuse congestion and proteinaceous exudate within atria and air capillaries.

11. A higher magnification of a section of lung from a MSD virus-infected bird (H&E, X100). In addition to the congestion and proteinaceous fluid, there are moderate numbers of histiocytes within the septa and lumens of atria and air capillaries.

12. Section of the same lung from an infected bird, stained with the streptavidin-biotin immunoperoxidase method (X64). There are moderate numbers of red-staining histiocytes, which indicates that MSD viral antigen is present within these cells. Turkeys infected with hemorrhagic enteritis virus do not develop pulmonary edema, as seen in MSD virus infection.

13. Section of liver from a MSD virus-infected pheasant contains a typical intranuclear inclusion in the center of the field. Intranuclear inclusions may be found scattered through the parenchyma of the liver, kidney, and bone marrow in some pheasants infected with MSD virus. Experimental production of MSD has demonstrated that these inclusions are transient and most frequently observed between 6 and 10 days post-inoculation.

14. Section of liver as in the previous slide, stained with the streptavidin-biotin immunoperoxidase method (X200). The single red inclusion contrasts markedly with the pale basophilic staining of the surrounding parenchyma.

15. Transmission electron micrograph of splenic tissue from a MSD virus-infected bird (X8,250). The cell in the center of the field is a reticuloendothelial cell with an enlarged nucleus, marginated chromatin, and intranuclear viral particles.

16. At higher magnification (X50,000), the non-enveloped, 70 to 90 nm diameter viral particles can be seen within the nucleus.

17. Photomicrograph of a suspension of MDTC RP-19 cells (X128), which is the only cell line in which it has been possible to propagate type II avian adenoviruses, including MSD virus. These cells are an immortal line of B lymphoblasts which were originally derived from turkey livers infected with Marek's disease. These cells require a complicated culture medium with several supplements and specialized incubation conditions. Therefore, MSD virus is rarely isolated by cell culture under routine diagnostic situations. Several currently available

commercial vaccines against MSD use virus which was propagated in RP-19 cell cultures.

18. MDTC RP-19 cells were inoculated with MSD virus approximately 72 hours prior to taking this photomicrograph (X128). Typical cytopathic effects include cell swelling and cytoplasmic blebbing. Eventually infected cells lyse, and the shrunken cellular remnants are indistinguishable from other aged RP-19 cells in the media.

19. Frozen section of MSD virus-infected splenic tissue which has been stained with an indirect fluorescent antibody technique (X160). The primary antibody was hyperimmune antisera against MSD virus, and the secondary antibody was an anti-turkey (or anti-chicken) IgG antibody of goat-origin labeled with fluorescein isothiocyanate (FITC). When examined under a fluorescence microscope, the specific yellow fluorescence indicates the presence of MSD viral antigen in the tissue section.

20. Another important laboratory test for the diagnosis of MSD is the agar gel precipitin assay pictured on this slide. While more sensitive enzyme-linked immunosorbency assays (ELISA) have been developed for serologic detection of MSD virus antibodies, the agar gel precipitin test remains the most widely used serologic test. Test sera are placed within the outer wells of the agar, while the center well contains a suspension of MSD virus-infected splenic tissue. Thin bands of precipitin form in the agar between the inner well and the outer wells, which contain positive sera. In this plate, a positive control serum

is present in the top well. Four additional wells contain positive sera, while a single well (2 o'clock position), without a precipitin band, contains negative serum. The agar gel precipitin test can also be performed using known positive serum in the center well, and placing suspensions of spenic tissue to be tested for MSD antigen in the outer wells. Testing splenic suspensions for virus antigen may be used for rapid diagnosis during an outbreak of MSD, while testing sera for antibody presence is most useful following seroconversion, several weeks after an outbreak.

SELECTED REFERENCES

Domermuth, C. H. and W. B. Gross. Hemorrhagic enteritis and related infections. In: *Diseases of Poultry*, 9th ed. B. W. Calneck, H. J. Barnes, C. W. Beard, W. M. Reid and H. W. Yoder, Jr., eds. pp. 567-572. 1991.

Fitzgerald, S. D. and W. M. Reed. A review of marble spleen disease of ring-necked pheasants. J. Wildl. Dis. 25:455-461, 1989.

Jakowski, R. M. and D. S. Wyand. Marble spleen disease in pheasants: demonstration of agar gel precipitin antibody in pheasants from an infected flock. J. Wildl. Dis. 8:261-163, 1972.

Nazerian, K. and A. M. Fadly. Propagation of virulent and avirulent turkey hemorrhagic enteritis virus in cell culture. Avian Dis. 26:816-827, 1982.

Wyand, D. S., R. M. Jakowski and C. D. Burke. Marble spleen disease in ring-necked pheasants: histology and ultrastructure. Avian Dis. 16:319-329, 1972.

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