Marble Spleen Disease of Pheasants in Ontario

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ABSTRACT

Two outbreaks of marble spleen disease were observed in pen-raised pheasants in Ontario on the same farm in the summer of 1971 and in 1972. In both instances, a mortality of 3% per day lasted for approximately 14 days. Fifteen birds were necropsied and tissues removed and examined histologically and by electron microscopy. In addition splenic material was injected into adult pheasants. The gross lesions, characterized by enlarged greyish-white mottled spleens and acute congestion and oedema in the lungs, were identical to those described by previous authors. Histological examination of livers, lungs, spleens and proventriculi revealed some reticular-type cells which contained either an eosinophilic or a basophilic intranuclear inclusion. Electron microscopy of the affected spleens revealed cells with massive intranuclear accumulations of virus particles. Although transmission trials failed, the evident histological and electron microscopy lesions confirm the suggestion by other authors that this is a viral disease affecting the reticulo-endothelial system.

RÉSUMÉ

A deux reprises, soit au cours des étés 1971 et 1972, la maladie de la rate marbrée a sévi chez des faisans élevés dans des parcs, sur une ferme de l’Ontario. Dans les deux cas, la mortalité atteignit 3% et dura environ 14 jours. On effectua la nécropsie de 15 sujets et on préleva des tissus en vue d’examens au microscope ordinaire et électronique. De plus, on injecta des suspensions de tissu splénique à des faisans adultes. Les lésions macroscopiques se traduisirent par une hypertrophie et une apparence marbrée gris-blanc de la rate, ainsi que par de la congestion aiguë et de l’œdème pulmonaires. Ces lésions s’avérèrent identiques à celles que d’autres auteurs ont déjà décrites. L’examen histologique du foie, des poumons, de la rate et du proventricule révéla la présence de cellules du type réticulaire, contenant des inclusions intra-nucléaires acidophiles ou basophiles. L’examen au microscope électronique de rates lésées révèle une accumulation massive de particules virales, dans le noyau de certaines cellules. Malgré l’échec d’essais visant à transmettre la maladie, l’histopathologie et la microscopie électronique confirment l’opinion d’autres auteurs, à savoir qu’il s’agit d’une maladie virale affectant le système réticulo-endothélial.

INTRODUCTION

Marble spleen disease (MSD) in pen-raised pheasants has been reported in Italy (2), Pennsylvania (3) and Connecticut (4).
The disease as described in these reports is characterized by enlarged, greyish-white mottled spleens and acute congestion and oedema of the lungs. This paper reports the observation of a syndrome in pheasants in Ontario, which appeared identical in gross, histological and electron microscopy lesions to those described by other workers.

The disease occurred in flocks of pen-raised pheasants on the same farm in Ontario in two consecutive summers — July of 1971, and 1972. In the 1971 outbreak, the owner reported that eight week old pheasants died suddenly with no apparent signs of previous illness, and that the mortality of 3% per day persisted for a period of about two weeks. The signs were the same in 1972 except the birds were twelve weeks old when affected. In both instances, the flocks were being fed a commercial pheasant feed.

**MATERIALS AND METHODS**

Complete necropsies were performed on fifteen birds received for examination. Portions of liver, lung, spleen, esophagus, brain, proventriculus, kidney, heart, pancreas and intestine were fixed in 10% buffered formalin and sectioned at six microns. These tissues were then stained with hemotoxylin-eosin, Thioflavine S, Congo red, crystal violet, for the periodic acid-schiff reaction and by Feulgen’s method.

Portions of the spleens were removed from the formalin, dehydrated in graded xylol solutions, fixed in glutaraldehyde and osmium tetroxide, embedded in epon, sectioned and examined under the electron microscope.

Lung, liver and spleen were cultured aerobically on MacConkey’s and blood agar media.

The entire spleens of several affected birds were minced and placed in 30 ml of distilled water, then shaken in a Waring blender for 15 minutes. Five ml of the resultant suspension was then injected intraperitoneally into each of three adult pheasants that had been reared in isolation.

**RESULTS**

Gross pathological examination revealed lesions mainly in the spleen and lung. The spleens were consistently enlarged with numerous uniform greyish necrotic lesions (Fig. 1). The lungs were intensely congested and very oedematous. An excess of

![Typical focal "marbling" of the enlarged spleen.](image-url)
frothy exudate was present in the infraorbital sinuses of some specimens, and in the birds observed in the 1972 outbreak, ulcerative lesions were observed in the intestinal tract of two of the ten birds.

Bacteriological examination of the spleens, livers and lungs revealed only a few Escherichia coli organisms which were not considered significant.

Attempts at transmitting the infection using splenic material were unsuccessful. The inoculated birds were observed daily for four weeks after inoculation during which time no illness occurred. Necropsies performed at the end of the period of observation failed to reveal any gross or histological lesions.

Histological examination of the lung sections of affected birds showed many areas of focal necrosis in the air capillary areas around the tertiary bronchi. A pink amorphous material in the necrotic areas contained a few nuclei showing eosinophilic and basophilic intranuclear inclusions. Some of the atria showed minor accumulations of heterophils and fibrin. Many other areas of the lung were found to be severely congested with hemorrhage into the tertiary bronchi.

The spleens revealed large areas of focal necrosis in the germinal follicle areas, with lymphoid cell necrosis and degeneration of reticular cells. Again, intranuclear inclusions of basophilic and eosinophilic complexion were seen commonly throughout the organ (Fig. 2). Margination of chromatin in all cells containing inclusions was common. The eosinophilic inclusions were numerous in both the lung and spleen. Amorphous eosinophilic material was observed in both the lungs and the spleen; however, this failed to react positively to amyloid with either Thioflavine S, or Congo red stain. The crystal violet stain revealed material suggestive of amyloid, and the electron microscopy examination revealed a fibrillar material; however, this was not identified as amyloid.

The liver contained eosinophilic inclusion bodies in the nuclei of some of the Kupffer cells. The livers were found to be very congested and some small areas of necrosis were present, usually in a periacinar arrangement.

In the proventriculus there were lymphocyte aggregates under the simple glandular layer, and a few eosinophilic inclusion bodies present in the nuclei of degenerate cells trapped in the glandular excretion. Focal areas of necrosis and inflammation were found in the pancreas and intestine, with lymphocytes and a few heterophils present in these organs. No histological lesions were found in the trachea, heart, eosinophagous, brain or kidney.

The electron microscopy examination of affected spleens revealed cells in which there were massive accumulations of virus particles in the nuclei, in some cases appearing to swell the nuclei to the point of rupture of the structure, and viral particles were seen outside the nuclear membrane (Fig. 3). The cells generally affected had oval shaped nuclei with elongated cytoplasm and appeared to be reticular-type cells. Three types of viral particles were evident — large vacuole-like particles approximately 110 mu in diameter with only an outer membrane, particles containing a

![Fig. 2. Spleen — Eosinophilic (A) and basophilic (B) intranuclear inclusions in cells of the germinal follicles of the spleen. H & E X566.](image-url)
loose osmiophilic substance in the centre of the vacuolar area; and mature virions with the central portion filled with a dense osmiophilic core. These latter structures have an approximate diameter of 90 μm. Many of the virus particles presented a profile suggesting a hexagonal structure (Fig. 4).

**DISCUSSION**

This paper reports the first cases of so-called marble spleen disease in Canada. The disease has been seen in several parts of the United States and Italy, and the histological and gross pathology, along with a demonstration of the viral particles, suggest that this is probably the same disease. Previous workers report lesions only in the spleen and lungs of the affected cases; however, in the outbreak described here, although there was no gross evidence of damage, there were evident histological lesions in the liver and proventriculus. There was periacinar necrosis and intranuclear eosinophilic inclusions in a few Kupffer cells. In the proventricular glands, although the cells could not be positively identified there were intranuclear eosinophilic inclusions in some cells in the sloughed debris in the lumen. The ulcerative intestinal lesions seen in only a small number of birds indicated this was an incidental finding.
Although crystal violet stain revealed material suggestive of amyloid and the electron microscope showed a fibrillar material, other more specific stains for this substance were negative. It was not possible to confirm that the substance observed was amyloid.

Wyand et al (4) observed virus particles in the splenic intranuclear inclusions about 70 μ in diameter with both empty and electron dense cores and suggested that the cubic symmetry and size range of particles indicate adenoviruses. In the electron microscopy observations made on the specimens described here, numerous particles were observed outside of the nuclei, with some aggregates loose in the cellular debris. The virus particles resemble those described by Wyand et al (4), but appear slightly larger and in greater numbers. The gradual

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Fig. 4. Electron micrograph of virus particles seen in spleen cell nucleus showing the three general particle types: (A) vacuole-like particles; (B) particles with loose osmiophilic substance in the core; (C) virions with central portion filled with dense osmiophilic core. X48,325.

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increasing density of the viral cores was assumed to be associated with progressive maturity of the virion. Attempts to classify the virus were not made.

Several workers attempted to reproduce the disease or isolate a virus. Rothenbacher et al (3), on oral and intraperitoneal inoculation of 20 pheasants produced the disease in one pheasant only. A similar experiment with 300 pheasants had negative results, and organ emulsions from field cases killed 50% of inoculated pheasant and chick embryos in seven days. The same material from the dead embryos when passed into other pheasant or chick embryos frequently induced either death or stunting. Mandelli et al (2) were unsuccessful in isolating a virus from liver or spleen from affected cases, nor could they reproduce the disease in pheasant or mice. Wyand et al (4) were unable to produce cytopathogenic effect by inoculation of infected spleen material on chicken embryo fibroblasts, chicken kidney cells or pheasant kidney cells. However, they detected agar gel precipitin antibody on 11% of pheasant serum samples collected from a flock which had experienced a 2% mortality from MSD three to four months earlier (1). They indicate that this finding substantiates their suggestion of a viral etiology for the disease. Unfortunately, circumstances prevented virological isolation attempts at the time the investigations reported here were under way, and transmission attempts in adult pheasants failed. However, the observations of profuse production of virus in the spleen cells suggest that these particles are involved in the disease syndrome.

Previous workers reporting this disease observed the disease in mature birds. In the outbreaks observed in Ontario, the birds were eight to twelve weeks of age which was considerably below the age at which others noted the disease.

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REFERENCES