Case Report—

A Case of Acute Pulmonary Edema, Splenomegaly, and Ascites in Guinea Fowl

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SUMMARY. Acute pulmonary edema, splenomegaly, and ascites were observed in a disease outbreak in adult white and pearl guinea fowl. The clinical history and gross and microscopic lesions resembled those described for marble spleen disease of pheasants and avian adenovirus group II splenomegaly of chickens. A small number of intranuclear inclusion bodies were found in liver, spleen, and lung sections of affected guinea fowl. Attempts to isolate virus and serological tests to detect the presence of viral antigens were unsuccessful.

Adult female pearl guinea fowl experimentally exposed to pheasant and turkey isolates of type II avian adenoviruses developed gross and microscopic lesions similar to those seen in the field outbreak. The pheasant isolate was the more virulent. Intranuclear inclusion bodies were observed in liver, spleen, and lung sections of pearl guinea fowl inoculated with either of the virus isolates, and direct immunofluorescent examination revealed viral antigen in the spleen and lung.

RESUMEN. Reporte de Caso—Edema pulmonar agudo, esplenomegalia y ascitis en gallinas de Guinea.

Se presentó un brote con edema pulmonar agudo, esplenomegalia y ascitis en gallinas de Guinea adultas blancas y pintadas. La historia clínica y las lesiones macro y microscópicas fueron semejantes a las observadas en la enfermedad del bazo moteado de faisanes y la esplenomegalia causada por adenovirus aviar grupo II en pollos. Se encontró un número pequeño de cuerpos de inclusión intranucleares en secciones de hígado, bazo y pulmón. No fructificaron los intentos para aislar el virus o para identificar los antígenos virales por medio de las pruebas serológicas.

Gallinas de Guinea pintadas expuestas experimentalmente a cepas del tipo II de adenovirus de faisán y pollo desarrollaron lesiones macro y microscópicas similares a las observadas en el brote de campo, siendo la cepa de faisán la más virulenta. Se encontraron cuerpos de inclusión intranucleares en secciones de hígado, bazo y pulmón de gallinas de Guinea pintadas inoculadas con cualquiera de las cepas y el examen por inmunofluorescencia directa identificó el antígeno viral en bazo y pulmón.

A literature search failed to reveal any reports of a guinea fowl disease resembling marble spleen disease (MSD) of pheasants (5) or avian adenovirus group II splenomegaly (AAS) of chickens (5). These diseases plus hemorrhagic enteritis (HE) of turkeys are caused by type II avian adenoviruses (1), and pheasants, chickens, and turkeys are the only known natural hosts (5). However, fowl adenoviruses (type I avian adenoviruses) (1) have been isolated by Pascucci et al. (15) from guinea fowl with pancreatitis.

No antibody to type II avian adenoviruses has been detected in wild bird sera (2). Laboratory studies have demonstrated that pheasant and chicken isolates of type II avian adenoviruses will infect turkeys and that turkey isolates will infect pheasants (3,6,7,12). Additionally, exper-
experimental HE virus infection of golden pheasant, peafowl, chickens, and chukars produced enlarged and mottled spleens but no mortality (5).

The following is a case report of acute pulmonary edema, splenomegaly, and ascites in adult guinea fowl. Results of experimental type II avian adenovirus infection of guinea fowl are also discussed.

CASE REPORT

An eastern Pennsylvania game farm experienced a sudden onset in mortality (33 of 1500; 2.2%) among white guinea fowl breeders in late April 1983. An egg-production drop of 30–50% was calculated for these breeders, which were maintained in four different houses. Collected eggs were of normal size, shape, and color. Approximately 1 week following the disease onset, pearl guinea fowl housed adjacent to the white guinea fowl were observed to be affected. No vaccines or antibiotics had been administered, and no significant disease problems had been encountered previously by either of these guinea fowl flocks.

Eight dead and four live guinea fowl hens were submitted to the Livestock and Poultry Veterinary Diagnostic Laboratory at The Pennsylvania State University for examination. Dead and sick birds were cyanotic, and a greenish diarrhea was observed on vent feathers. Several birds had distended abdomens which contained copious amounts of straw-colored fluid. Affected hens appeared to be of normal weight and were ovulating, but the ovarian follicles were hemorrhagic, and some had ruptured, causing egg-yolk peritonitis. The lungs were edematous, and spleens were enlarged (2–4×) and mottled (Fig. 1); lesions were similar to those described for MSD and AAS (5). The lungs also exhibited acute

Fig. 1. Lungs with acute edema; enlarged and mottled spleens.
congestion, and serosanguineous fluid exuded from the cut surfaces. Livers were swollen, and some had areas of greenish discoloration on the surface. Most hens had a mild to moderate catarrhal enteritis, which was not grossly hemorrhagic. The intestinal walls of several hens were thickened, and cecal worms were present.

Histopathologic examination of hematoxylin-and-eosin (H&E)-stained sections of spleen showed characteristic multifocal histiocytic hyperplasia and necrosis. The pulmonary lesion was acute congestion and edema (Fig. 2). Periportal hepatitis and cholangitis with multifocal necrosis was observed in the liver. Nuclear changes resembling those of type II avian adenovirus infections (nuclear vesiculation and inclusion body formation) were seen in histiocytes of the spleen and lung (Fig. 2) and in hepatocytes (Fig. 3). The enteritis ranged from catarrhal to erosive-hemorrhagic. Capillaria cross-sections were found in the mucosa. Glomerular and tubular nephritis was present in the kidneys. No brain lesions were detected. Special staining techniques (Jenner-Giemsa and Warthin-Starry) for spirochetes revealed nothing.

A hematological evaluation of four affected guinea fowl hens revealed a relative lymphopenia, monocytosis, and heterophilia. Red blood cells had hypochromic nuclei and immature shapes.

Bacterial culturing of liver sections, abdominal fluid, and swabs of the abdominal cavity of both dead and moribund birds resulted in a mixed flora of *Escherichia coli*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, and *Bacillus* sp. Because of the suspected viral nature of this disease, acute and convalescent sera were evaluated by double immunodiffusion (DID) (4) and enzyme-linked immunosorbent assay (11) and were found to be free of reovirus, reticuloendotheliosis virus (REV), and type I and II avian adenovirus antibodies (1,4). Spleens were examined for the presence of type II avian adenovirus antigen by DID and direct immunofluorescence (DIF; fluorescein isothiocyanate conjugate) (8) and were also found to be negative. An attempt to isolate a type II avian adenovirus from spleen homogenates was made in a B-lymphoblastoid cell line (MDTC-RP19) (13,14) with no success. Attempts to isolate other viruses by yolk-sac inoculation of specific-pathogen-free (SPF) chicken embryos (10) and inoculation of SPF chicken-embryo-liver-cell culture (16) were also unsuccessful.

In an effort to obtain additional information about the etiology of the above described disease, three clinically normal 18-week-old pearl guinea fowl hens were inoculated intravenously (0.5 ml) with approximately $5 \times 10^4$ TCID$_{50}$ of pheasant or turkey type II avian adenoviruses. Two hens
(representing one of each inoculum type) were sacrificed at 4 days postinoculation for pathological evaluation. No clinical signs were observed before euthanasia. One hen was held for assays of antibody stimulation.

Sacrificed hens exhibited distended gas-filled intestines and ceca, enlarged marbled spleens, and congested edematous lungs (pheasant virus only). Gross lesions were more marked in the pheasant virus (PV)-inoculated hen. Microscopic lesions were similar to those described for the reported field outbreak and were more intense in the guinea fowl exposed to the PV isolate. The spleen of the PV-inoculated fowl
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exhibited multifocal necrosis, and nuclei of histiocytes were found to be vesiculated and to contain amphophilic inclusion bodies. Acute congestion and mononuclear pneumonitis was detected in the lung of the PV-inoculated fowl. Pulmonary histiocytes had vesiculated nuclei with eosinophilic to lavender to slightly basophilic inclusions (Fig. 4). Cholangiohepatitis was seen in the liver of the PV-inoculated fowl. Numerous hepatocytes had enlarged nuclei with eosinophilic to lavender inclusion bodies (Fig. 5). The pancreas of this bird had foci of hydropic degeneration.

The histopathologic findings for the turkey-virus-infected guinea fowl were similar to but less intense than those found in the PV-inoculated guinea fowl; there was less necrosis and fewer vesiculated nuclei of histiocytes with inclusion bodies. The microscopic lesions observed in the intestine and kidney of experimentally infected guinea fowl (both viruses) were essentially the same as those found in the field case.

Viral protein was detected by DIF in the spleen and lung of experimentally infected guinea fowl (both virus isolates), which supports the gross and microscopic findings. Weekly assays for precipitin formation in guinea fowl experimentally inoculated twice (3 weeks apart) with the PV isolate were negative through 3 months post primary inoculation.

**DISCUSSION**

The gross and microscopic lesions in the spleens and lungs of white and pearl guinea fowl plus the clinical history of this case are similar to those described for MSD of pheasants (5) and AAS of chickens (5).

The histopathologic changes seen in guinea fowl spleens (field case and experimental) included lymphocytic depletion and proliferation and necrosis of histiocytes. The type of intranuclear inclusion bodies observed in splenic histiocytes have been described as pathognomonic for MSD of pheasants (18). Additionally, these guinea fowl exhibited an acute pneumonic congestion and edema, which closely resembled signs described for MSD and AAS (5).

Although no virus could be isolated and no viral protein could be detected by DID and DIF in guinea fowl spleens from the field outbreak, the pathologic and DIF findings of field and/or experimental cases are suggestive of a “type II avian adenovirus splenomegaly” of guinea fowl. The hematology and renal histopathology are also suggestive of a viral etiology. Since the existence of virus and/or viral protein in type II avian-adenovirus-infected birds (9,17) is transient, it is understandable that virus might be missed in field cases. Type II avian-adenovirus-induced precipitin could not be detected in either field or experimentally infected guinea fowl. In retro-
spect, one additional virus-isolation technique should have been attempted: the inoculation of SPF turkey poults with the intestinal contents and/or minced splenic tissue of dead or morbund guinea fowl (4,6).

More research is needed to clarify the etiology of the described guinea fowl disease.

REFERENCES


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