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Natural Occurrence and Experimental Study of Pox and *Haemoproteus*Infections in a Mute Swan (Cygnus olor)

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Abstract

Pox and *Haemoproteus sp.* infections in a mute swan are reported. Experimental study of the swan pox isolate indicated that geese were susceptible, but chickens, ducks, and pigeons were resistant to the virus. The distinctive feature of pox infection in the swan was the invasive proliferation deep into the subcutaneous tissues of the face and beak, producing facial deformity and swelling.

Introduction

Fowl pox infection has not been reported in swans (subfamily *Cygninae*), although outbreaks have been reported in other members of the family *Anatidae*. The validity of some reports is questionable. Although Ward and Gallagher¹⁷ stated that pox occurs naturally among geese and ducks, and Te Hennepe¹⁶ (cited by Doyle and Minett⁸) reported outbreaks in ducks, supporting diagnostic criteria were not presented. Jansen⁸ is of the opinion that Te Hennepe incorrectly interpreted lesions of duck plague as fowl pox. Ratcliffe¹⁴ reported lethal pox infection of 2 American Scoters (*Oidemia nigra americana*) in the Philadelphia Zoo.

Rao¹³ confirmed outbreaks of pox in domestic ducks, demonstrated specific inclusion bodies, and isolated the virus. He found the isolate host-specific, and was unable to infect chickens or pigeons. Doyle and Minett³ and Irons⁷ were unable to infect ducks with pox strains of either chicken or pigeon origin. Kirmse¹⁰ reported experimental infection of ducks and geese by the intravenous and intradermal administration of fowl pox virus; however, he was unable to demonstrate pox inclusion bodies consistently.

The following is a report and experimental study of pox and *Haemoproteus* infections in a Mute Swan.

History and Necropsy Findings

On August 3, 1964, a dead five-week-old mute swan was submitted to the laboratory for examination. The cygnet was one of a spring brood of five that inhabited a small stagnant cove of the Long Island Sound on the north shore of Long Island adjacent to the town of Northport. This area was heavily infested with mosquitoes. The specimen was decomposed and covered with fly maggots.

On the following week, the second swan of the same brood was submitted to the laboratory. The bird was alive, but unable to walk or stand. The bird was sacrificed, and necropsy findings revealed a slight aerosacculitis, grey and bronze liver mottling, and pale kidneys. A few unsporulated *Tyzzeria*-like oocysts were noted in wet smears taken from the intestinal mucosa. Bacteriologic cultures of the heart blood, liver, spleen, and gall bladder yielded Staphylococci. Brain cultures were negative.

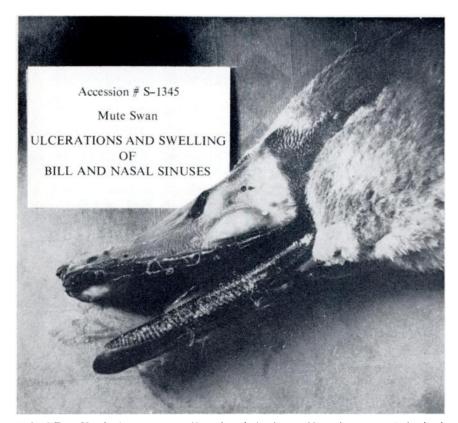


FIGURE 1. Head of mute swan affected with fowl pox. Note distortion of the beak and swelling.

Five weeks following the initial accession, the third swan of the brood was submitted to the laboratory. The bird was dead and partially decomposed. The beak, face, and oral cavity were greatly deformed, with multiple elevated confluent nodules and scabs (Fig. 1). These lesions extended into the nasal sinuses. Macules, papules, scabs, and ulcers were noted on the unfeathered portions of the skin, especially on the foot web, where crater-like ulcers ranging in size from approximately 0.5 to 1.5 cm in diameter were noted. Selected scabs and nodules were removed for virus isolation attempts.

Microscopic examination revealed the invasive tumor-like character of the head lesions, which extended deep into the subcutaneous structures as parallel lobulated cords of affected epithelial cells. The acidophilic intracytoplasmic Bollinger bodies were more abundant in the deeper tissues.

In contrast, lesions of the foot web were found to be more superficial, less proliferative, with greater surface area involvement. The latter changes ranged from areas of limited superficial focal necrosis to extensive dishedout surface defects (ulcers). Inclusion bodies were present also in the deeper margins of these lesions.

The opened carcass revealed a grey liver surface with pinpoint white spots, white miliary areas on the epicardium, and a hemorrhagic enteritis.

Bacteriologic cultures of the heart blood, liver, spleen, and ovary yielded *E. coli*. Sections of the heart, liver, spleen, kidney, lung, beak nodules, web skin, and brain were taken for histopathologic study.

Examination of giemsa-stained heart blood smears revealed a light haemosporidial infection of the red blood cells. Although a few unidentified inclusions were observed in red blood cells, only gametocytes were recognized. The latter were identified as *Haemoproteus sp.*

The female gametocytes were dark, blue-staining, subspherical, spindle-shaped or globular forms (Fig. 2). The pink irregularly shaped

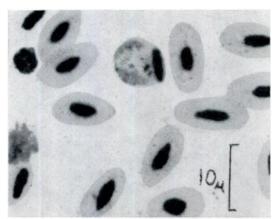


FIGURE 2. Haemoproteus female gametocyte in giemsa-stained blood smear.

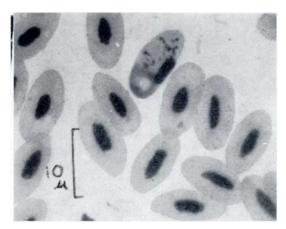


FIGURE 3. Haemoproteus male gametocyte in giemsa-stained blood smear.

nucleus was difficult to define beneath the pigmented granules of the parasite. The nucleus of the host's red blood cell was usually displaced outwardly and was elevated above the remaining host cell wall. Occasional female gametocytes were noted free of their host cell investments. Measurement of 25 female gametocytes indicated an average width of 8.6μ and length of 10.4μ . The length ranged from 7.9 to 13.9μ , and the width from 6.4 to 9.9μ . There was an average of 21 pigmented granules for each female gametocyte.

The male gametocytes (Fig. 3) were a light blue color, larger and more elongated than the females, with fewer and less dispersed pigmented granules and less displacement of the red blood cell nucleus, which was moved laterally but did not project above the cell surface. The nucleus of the male gametocyte tended to be a pink central triangular area, and usually a dark blue circular band was noted at one pole. Measurement of 25 male gametocytes averaged 8.7 x 14.3μ . The length ranged from 11.8 to 19.8μ . Width ranged from 7.9 to 9.9μ . There was an average of 15 pigmented granules per parasite.

Materials and Methods

The beak and skin nodules removed at necropsy were homogenized, and antibiotics were added to the supernatant fluid, which was employed as the inoculum for attempted virus isolation. One-half ml of the inoculum was placed upon the chorioallantoic membrane (CAM) of chicken eggs on the 12th day of incubation. Of the six embryonating chicken eggs thus inoculated, death occurred in two. One died five days and the second died eight days postinoculation. All of the CAM were found to have diffuse fowl pox lesions within and around the area of inoculation at the time of embryo death, or on the ninth day when the viable embryos were sacrificed. Bollinger and Borrel bodies were observed in the membrane lesions.

Four 6-month-old White Embden geese, five 5-week-old White Pekin ducklings, and five 30-week-old White Leghorn chickens were selected from confinement-reared laboratory flocks which had had no known outbreaks or exposure to fowl pox virus. Five confinement-reared 4-month-old domestic pigeons were obtained from a pigeon broiler flock that had no previous history of pox.

Two of each of the above species were placed in separate pens and served as uninfected controls. The remainder (two geese, three chickens, three pigeons, and three ducks) were inoculated with the original suspension prepared from the swan pox lesions. The inoculum was brushed into scarified skin areas of the beak, face, wing web, and foot web (geese and ducks) or interdigital tissue (pigeons and chickens). In addition, the nasal mucosa was scarified and the inoculum brushed onto the exposed surface and allowed to pass into the nasal passage. The exposed birds were placed in separate pens according to species in the same room as the unexposed control birds.

All birds were examined daily for a 30-day period following this inoculation. When skin lesions were observed, a portion of the affected tissues was removed for attempted virus isolation and histopathologic study.

At the end of the observation period, all birds (including the controls) which had failed to develop lesions were reinoculated in the same location and by the same method with a recent field isolate of fowl pox virus from chickens. All birds were observed for a further 30 days. Samples of affected tissues were removed for study.

Results

Following the inoculation with the swan pox suspension, only the geese evidenced lesions of pox. The lesions began to appear five days postinoculation, and became progressively larger. The lesions, however, were more limited when compared to those observed in the swan and did not produce deformity of the head structures. Lesions consisted of macules, scabs, and nodules at all of the sites of inoculation. Histopathologic study of the biopsied lesions revealed very few Bollinger bodies. The virus was reisolated from these lesions, and inclusion bodies were demonstrated in the CAM of the inoculated chicken eggs employed for virus isolation. These infected geese were not employed in the remainder of this study.

Following challenge with the field isolate of fowl pox from chickens, only the chickens developed lesions of pox. Both the uninoculated and swan pox exposed chickens developed pox lesions at five days after exposure. The virus was reisolated from these pox lesions using the CAM of embryonating chicken eggs. Bollinger bodies were demonstrated in the affected CAM and in the pox nodules from the chicken.

Discussion

Of the Haemosporidia described from swans, Coatney and Roudabush, Herman, '.s.' and Lapage" cite the following references for species of swans:

Cygnus atratus (Latham), Black Swan Plasmodium (Proteosoma) bizurae^a Plasmodium biziurae^a Plasmodium sp.^a Cygnus columbianus columbianus (Ord), Whistling Swan Haemoproteus sp. 4.5.18

Cygnus melanocoryphus (Molina), Black-necked Swan Plasmodium relictum¹⁵
Plasmodium sp.²

Cygnus olor (Gmelin), Mute Swan Haemoproteus sp. 6

Herman observed the latter infection in captive mute swans in Connecticut and New Jersey during the year 1938. Since none of the life cycles has been established, there is some doubt as to the validity of the species of Haemosporidia described from swans.

Because of the limited and possibly misleading reports of Haemosporidia of swans, species identification of the *Haemoproteus* observed in the swan could not be made, but must await future definition.

The frequency of natural occurrence of fowl pox in swans is unknown, and this report confirms their susceptibility. Pox should be considered as a possible cause of mortality in swans. The distinctive feature of the disease in this instance, and as reported by Rao¹³ in ducks, is the deep invasive growth in the subcutaneous tissues of the head region, resulting in deformity and facial swelling. Such lesions of the beak and face may be indicative of pox in Anseriformes. Although Ratcliffe' suggests that such invasive pox growth are possibly induced by exposure to oil, it is likely that this effect is the characteristic pox response of Anseriformes. In addition, Rao¹³ noted two other manifestations of pox in ducks, namely, an "ocular" and "mouth" form.

Since only two geese were exposed to swan pox in this experimental study, only limited interpretation of the generalized response of geese can be made. The study does establish that geese were susceptible to swan pox and were resistant to the field strain of pox isolated from chickens. Since the swan-pox-infected geese were not rechallenged with the field isolate of pox from chickens, the protective immunity produced by swan pox in geese could not be evaluated. The manifestations of swan pox in geese were relatively mild when compared to the invasive growths noted in the swan. In addition, as was the case with Kirmse, 10 pox inclusions were difficult to demonstrate because of their limited numbers in the affected tissues of experimentally infected geese. However, the virus was reisolated from these tissues, and inclusion bodies were easily found in the CAM of embryonating chicken eggs employed for reisolation. The question is raised in this instance whether the virus infecting an alien host is inhibited in its normal inclusion body formation. A possibly related phenomenon is reported for rabies "fixed" virus, in which the inclusion body formation has been inhibited by continued serial passage of the virus through the central nervous system.12

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