Case Report—

Isolation of Poxvirus from Debilitating Cutaneous Lesions on Four Immature Grackles (Quiscalus sp.)

Douglas E. Docherty, A Renee I. R. Long, A Edward L. Flickinger, B and Louis N. Locke A

A U.S. Fish and Wildlife Service, National Wildlife Health Research Center, 6006 Schroeder Road, Madison, Wisconsin 53711
B U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Gulf Coast Research Station, P.O. Box 2506, Federal Building, Victoria, Texas 77902

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SUMMARY. Poxvirus was isolated from nodules on four immature grackles (Quiscalus sp.) collected in two residential areas of Victoria, Texas. All of the birds were emaciated and had nodules on the eyelids, bill, legs, toes, and areas of the skin on the wings, neck, and ventral abdomen. These pox nodules were extensive and probably interfered with both sight and flight. The preliminary diagnosis was confirmed by virus isolation, histopathology, and electron microscopy. Poxvirus was isolated on the chorioallantoic membrane of embryonated hen’s eggs and in Muscovy duck embryo fibroblast cell culture. Phaenicia calliborridae (blowfly) larvae were found in one of the pox nodules, raising the possibility of mechanical transmission of the virus by contaminated adult blowflies.

RESUMEN. Reporte de Caso—Aislamiento de virus de viruela a partir de lesiones cutáneas debilitantes de cuatro estorninos inmaduros (Quiscalus sp.).

Se aisló virus de viruela a partir de nódulos presentes en cuatro estorninos inmaduros (Quiscalus sp.) capturados en dos áreas residenciales de Victoria, Texas. Las cuatro aves estaban emaciadas y presentaron nódulos en los párpidos, pico, patas, dedos, y algunas áreas de la piel de las alas, cuello, y región inferior del abdomen. Estos nódulos producidos por viruela eran extensos y probablemente interfirieron con la visión y el vuelo. El diagnóstico presuntivo fue confirmado mediante aislamiento viral, histopatología, y microscopía electrónica. Se aisló el virus de viruela en la membrana corialantoidea de huevos embrionados de gallina y en cultivos celulares de fibroblasto de embrión de pato. Se encontraron larvas de mosca azul (Phaenicia calliborridae) en uno de los nódulos de viruela, incrementándose así la posibilidad de transmisión mecánica del virus mediante moscas adultas contaminadas.

The cutaneous form of avian pox infection has been reported in many wild bird species (5). A nodule on the foot of a common grackle (Quiscalus quiscula) was diagnosed histopathologically as avian pox by Herman et al. (3). Virus isolation was not attempted, and the infection was not reported to be debilitating.

In some cases, the location and/or size of the pox lesion can cause severe debilitation. The ponderance of pox lesions on the eyelids may contribute to death because the bird may not be able to find food (4) or escape predators.

The present report is on the first isolation of poxvirus from grackles (Quiscalus sp.) with debilitating pox lesions affecting both sight and flight.

MATERIALS AND METHODS

Case history. In August and September 1989, four immature grackles with pox-like lesions were submitted to the National Wildlife Health Research Center (NWHR) for diagnosis. The birds had been collected in two residential areas 4 km apart in Victoria,
Poxvirus in grackles

Fig. 1. Location of pox lesions on an immature grackle.

Texas. When found, all four of these birds were emaciated and incapable of normal flight. Five other immature grackles with similar nodules on their heads and necks but capable of normal flight also were observed at the sites.

Two species of grackles were observed in the area at the time of collection, the boat-tailed grackle (*Quiscalus major*) and great-tailed grackle (*Quiscalus mexicanus*). The smaller common grackle (*Quiscalus quiscula*) was not observed. Eye color is a key factor in distinguishing between adult boat-tailed and great-tailed grackles. However, the young of both species have the same eye color, making identification difficult (6). Consequently, we have referred to these immature grackles only as *Quiscalus* sp.

Cutaneous pox nodules were found at the commissure of the eye and bill; on the legs and toes; and on areas of the skin on the wings, neck, and ventral abdomen (Fig. 1). The nodules ranged from barely visible (½ mm wide by 1 mm high) to a large pendulant mass (25 mm wide by 21 mm high by 32 mm long). This largest of nodules was attached to the left wing and contained four *Phaenicia calliphoridae* (blowfly) larvae.

**Laboratory procedures.** A 10% suspension of each excised pox nodule was prepared in a viral transport medium consisting of Hanks' balanced salt solution (HBSS) with 0.5% glycerol and 100 μg gentamicin, 1500 IU penicillin, 1500 μg streptomycin, and 100 IU nystatin/ml. They were blended and centrifuged at 200 × g for 30 minutes. The supernatant was inoculated onto the chorioallantoic membrane (CAM) of embryonated chicken eggs (7) and Muscovy duck embryo fibroblast (MSDEF) monolayers (1) in Medium 199 with 10% gamma globulin-free fetal bovine serum. Cutaneous pox lesions from the birds and the CAM lesions were fixed in 10% buffered formalin, sectioned, and stained with hematoxylin and eosin. A 10% suspension of infected CAM was prepared in HBSS, blended, and centrifuged at 200 × g for 30 minutes, and the supernatant was centrifuged at 35,000 × g for 20 minutes. The resulting pellet was resuspended in distilled water, negatively stained with 4% phosphotungstic acid, and examined with an H500 Hitachi electron microscope.

**RESULTS AND DISCUSSION**

Intracytoplasmic inclusion bodies characteristic of poxvirus were observed microscopically in the cutaneous pox lesions and 7 days postin-
occlusion in the CAM. Viral cytopathic effect first appeared in MSDEF at about 2 days and progressed to 100% infection in 7 days. Cytopathic effect consisted of general cell rounding and was followed by degeneration. Numerous pox virions (325 \times 250 \text{ nm}) were observed with the electron microscope in the negatively stained preparations from infected CAM's (Fig. 2).

The emaciated condition of the grackles, typical of poxvirus infection, may have resulted from difficulty in finding food because pox lesions obstructed vision. This emaciated condition could also have contributed to the inability to fly normally. In the grackle with the large wing lesion, the physical presence of the lesion may have caused problems in flying. These pox lesions may not have been severe enough to cause death in all cases, and the lesions may have regressed as they do in some fowlpox-infected chickens (4), with the recovered birds being immune (2) as they matured into adulthood. This may account for the lack of observed poxvirus-infected adults at the collection sites.

Poxvirus is very persistent in the environment, and cutaneous infection occurs by direct contact with infected individuals or by mechanical transmission of the virus to broken skin. Skin of young birds can be traumatized when they are fed by adults or when they fight with other nestlings. The presence of Phaenicia callichoridae larvae in one of these pox nodules indicates that blowflies are attracted to the broken skin and will deposit their eggs in it. The poxvirus-contaminated insect (poxvirus was demonstrated in this lesion by histopathology and virus isolation) could then visit another bird with a broken skin area and mechanically transmit the virus.

REFERENCES


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