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## Host Specificity and Long Persistence of Pox Infection in the Flicker (*Colaptes auratus*)

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### ABSTRACT

The persistence of cutaneous lesions of avian pox virus infection in a yellow-shafted flicker (*Colaptes auratus*) over a period of 13 months is described. Extensive transmission experiments revealed strict host specificity for the flicker virus. Flicker-to-flicker transmission was achieved, both by inoculation and by cage contact. All attempts to isolate the virus were unsuccessful. The diagnosis of pox virus infection was confirmed by electronmicroscopy.

### INTRODUCTION

Pox in wild birds occurs more frequently than reports in the past have indicated. Though this disease often is present in game birds<sup>7-9</sup>, and smaller birds belonging to the finch family (*Fringillidae*)<sup>14</sup>, it has been reported only once in woodpeckers<sup>17</sup> and no attempts were made to isolate the virus.

In the course of trapping and banding birds in Southern Ontario during 1965-66, several yellow-shafted flickers (*Colaptes auratus*) of the woodpecker family (*Picidae*) were found with warty cutaneous lesions on their feet, beak, and around their eyes. Since the possibility existed that wild birds could disseminate pox disease among domestic birds, this study was undertaken to isolate this new strain of avian pox virus and to determine its transmissibility and pathogenicity to other wild and domestic birds.

### Material and Methods

Wild birds, trapped during bird banding activity in several areas in Southern Ontario,

were kept in large aviaries. At first woodpeckers were fed exclusively on mealworms but later they became adapted to a special food for insectivorous birds (Hykro Universal Food, made in Denmark). Biopsies were taken from cutaneous lesions and prepared for histopathology by fixatoxin in 10% buffered formalin and paraffin embedding. Sections were stained with haematoxylin-eosin, haematoxylin-phloxine-saffron, or periodic-acid-Schiff. Tissue biopsies were fixed by glutaraldehyde and osmium tetroxide and imbedded in "Mara-glass" for electronmicroscopy<sup>13</sup>. Other portions of lesions were ground with buffered saline, pH 7.2, containing 750 units penicillin, 750 mg neomycin and 750 mg streptomycin per ml in Tenbroeck tissue grinders. This was used for transmission experiments and for virus isolation studies. Natural transmission from diseased to healthy flickers was attempted by housing them in the same cage. Experimental transmission was attempted by inoculation of 0.5 ml of a 10% suspension of infected tissues. Generally the intradermal route was used (into the eyelids, skin of the plucked scalp, mucous membranes, feet and legs) but some intravenous and intramuscular injections were given. For attempted isolation of the virus, the choriolantoic membrane technique with 9 and 13 day old embryonated chicken eggs was used<sup>11</sup>. Attempts were made to propagate the flicker virus in roller tube cultures of chicken embryo tissues<sup>1</sup>.

Additional flicker specimens with pox infection were found in the skin collection of the Royal Ontario Museum.

#### RESULTS

During 1965-66 six cases of pox infection in adult flickers were investigated. Each case had pronounced lesions, mainly confined to the feet (fig. 1), but the mucous membranes of the mouth, the commissures of the mandibles, the base of the beak (where most of the pecking was observed), the skin on the forehead, the nasal chambers and the eyes (Table 1) were also involved. In two instances unilateral keratitis resulted in temporary blindness but in two to three weeks sight was restored. Captive flickers were observed for at least a year, and the progress of the infection could be followed. In birds exposed by contact the incubation period was approximately one month, after which the first lesions appeared, which in most cases sloughed after three months. One infected male flicker had persistent warty growths on the left foot for 13 months. Inclusion bodies could be demonstrated histologically in the lesions. After surgical removal of the lesions at 13 months, no lesions appeared during a further two months observation period.

Histological examination of the foot lesions showed abnormal proliferation of epithelial cells containing numerous aci-



Fig. 1 Foot lesions of a flicker with natural avian pox infection.

dophilic intracytoplasmic inclusion bodies (fig. 2 and 3). The yellowish caseous material found in diphtheritic patches of the mucous membranes of the mouth (fig. 4), in the nasal chambers or adherent to the subcutis of the eyeball, was mainly necrotic tissue containing colonies of bacteria. In some sections cells containing inclusion bodies were seen. Secondary invasion with bacteria and fungi was common, especially in lesions subjected to repeated biopsies.

Table 1. Natural Avian Pox Infection in the Flicker (*Colaptes auratus*)

| Sex | Date and Location           | Observation Period | Lesions                                                               | Keratitis |
|-----|-----------------------------|--------------------|-----------------------------------------------------------------------|-----------|
| M   | 23-4-65 L.P.                | 15 months          | both feet, mouth, mandibles                                           | left eye  |
| F   | 25-4-65 L.P.                | 3 days             | both feet, dorsum, beak, right eyelids, right nostril, nasal chamber  | no        |
| M   | 30-4-65 Toronto             | 17 days            | both feet, right hock, base beak, right mandible, forehead, eyesocket | right eye |
| M   | 30-4-66 L.P.                | 3 hours            | both feet cherry sized                                                | no        |
| F   | 18-9-27 Pottagville, R.O.M. |                    | left foot                                                             | no        |
| F   | 11-8-28 Pottagville, R.O.M. |                    | right foot                                                            | no        |

L.P. = Long Point Peninsula, Lake Erie

R.O.M. = Royal Ontario Museum

L.P. = Long Point Peninsula, Lake Erie

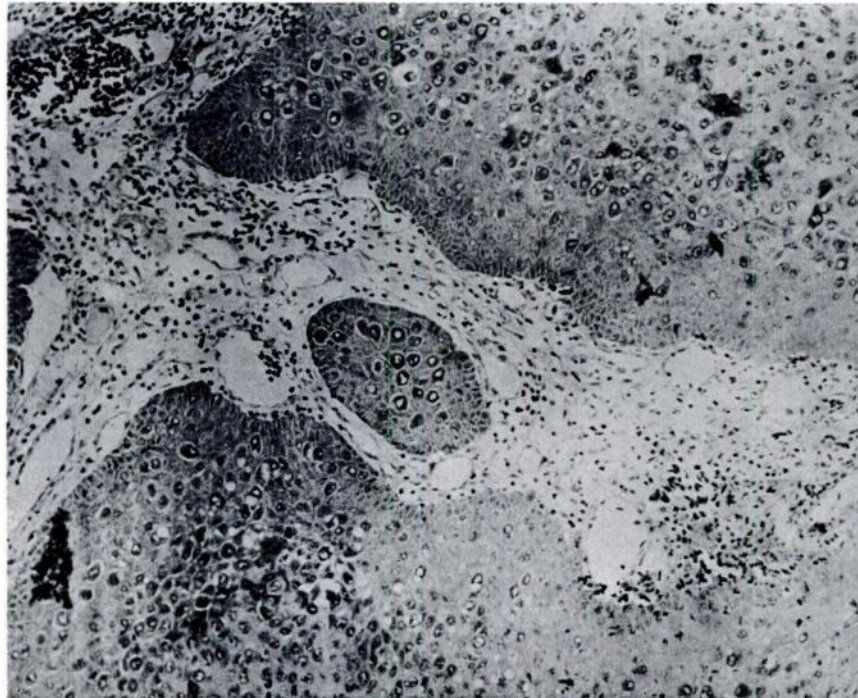


Fig. 2 Avian pox lesions from a naturally-infected flicker with hyperplastic epithelial cells containing vacuoles and acidophilic intracytoplasmic inclusions Hematoxylin-phloxine-saffron. X 180.

These infections seemed to be localized and had little effect on the birds' general health. Secondary mycosis has been reported also by others.<sup>7 18</sup> (Locke, Wirtz and Brown, 1965; DuBose, 1965).

Examination of flicker skins from the Royal Ontario Museum revealed in one the typical inclusions of avian pox as described above, but in the other only necrotic tissue and the condition of the tissue did not permit a definite diagnosis.

The electron micrographs (fig. 5) showed a distinct matrix in the inclusion body in which were embedded a large number of brick-shaped virus particles, 150 to 200 millimicrons in width and 265 to 350 millimicrons in length. A few particles were seen also in the surrounding cytoplasm. Intranuclear inclusions, as reported from another wild bird pox strain<sup>2</sup>, were not found.

#### Virus Isolation

Repeated attempts to propagate the flicker virus on the chorioallantoic membranes of embryonated chicken egg and in chicken embryo tissue cultures were unsuccessful, but a variety of pox strains from other wild and domestic birds were successfully isolated and propagated (unpublished).

#### Transmission Experiments

Two flickers were infected with the flicker virus (Table II), one by cage contact through pecking, in which the first lesions were observed 23 days post exposure; the other by inoculation of flicker material, in which lesions developed after one month. A third flicker was resistant to all routes of exposure (Table II). Both infected flickers were re-challenged with the flicker virus two

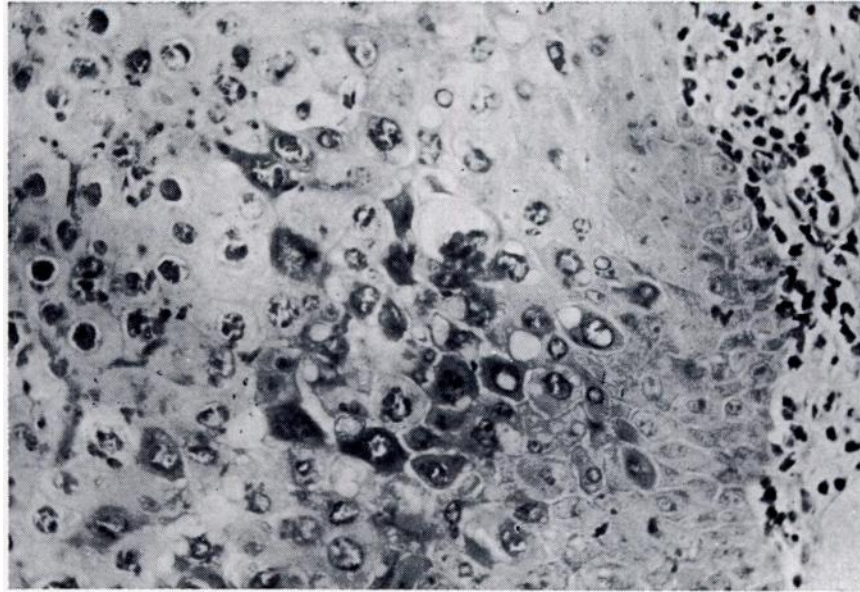


Fig. 3 Portion of fig. 2 enlarged, showing the transition from normal to diseased epithelial cells containing large vacuoles and one or more fine granular inclusions. X 725.

months after the first lesions had disappeared. The first case had developed an immunity which was also effective against exposure to a pox virus virulent for pigeons. The second case was infected and pinhead-sized nodules appeared on the oral mucous membranes and at the angles of the mouth. This successful reinfection was confirmed by histopathologic examination of the lesions. Attempts to infect numerous wild and domestic birds and mammals with the flicker virus were unsuccessful (Table III).

#### DISCUSSION

The cutaneous lesions of most pox virus infections are not of long duration.



Fig. 4 Pox lesion on the upper palate of a flicker with avian pox infection.

In smallpox, scabs slough within three weeks. In contagious pustular dermatitis, molluscum contagiosum, or paravaccinia, lesions can be present up to two

Table 2. *Experimental Avian Pox Infection In The Flicker (Colaptes auratus)*

| Sex                | Date and Location | Observation          | Incubation | Route of Exposure              | Lesions           | Immunity |
|--------------------|-------------------|----------------------|------------|--------------------------------|-------------------|----------|
| M                  | 24-4-65 L.P.      | 15 months            | 23 days    | contact                        | right foot, mouth | no       |
| F                  | 12-9-65 L.P.      | 8 months             | 30 days    | i.m., s.c., i.d.               | mouth, mandibles  | yes      |
| F                  | 10-10-65 L.P.     | 9 months             |            | i.d., i.v., i.m., s.c. contact | none              | yes      |
| i.d. = intradermal |                   | i.m. = intramuscular |            | s.c. = subcutaneous            |                   |          |



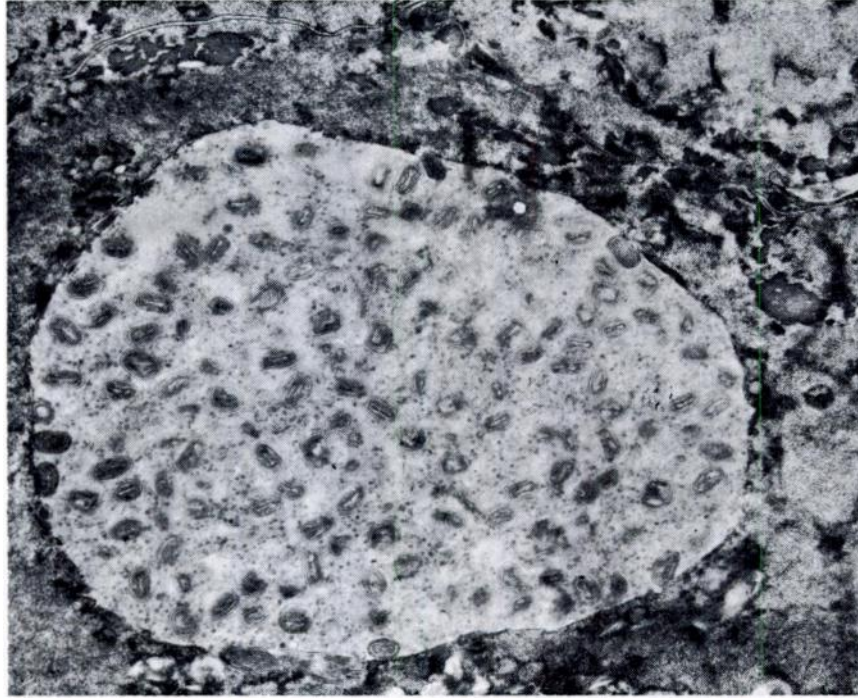


Fig. 5 Electronmicrograph of avian pox lesion of a flicker. Numerous virus particles are embedded in the matrix of the inclusion body (light area). X 34,400.

| Table 3. A list of Birds and Mammals Exposed to Flicker Pox Virus |                               |                    |
|-------------------------------------------------------------------|-------------------------------|--------------------|
| Common Names                                                      | Scientific Names              | No. of Individuals |
| <b>Wild Birds</b>                                                 |                               |                    |
| yellow-shafted flicker                                            | <i>Colaptes auratus</i>       | 3                  |
| redwinged blackbird                                               | <i>Agelaius phoeniceus</i>    | 15                 |
| robin                                                             | <i>Turdus migratorius</i>     | 14                 |
| cowbird                                                           | <i>Molothrus ater</i>         | 2                  |
| slate-colored junco                                               | <i>Junco hyemalis</i>         | 3                  |
| starling                                                          | <i>Sturnus vulgaris</i>       | 20                 |
| chipping sparrow                                                  | <i>Spizella passerina</i>     | 2                  |
| white-crowned sparrow                                             | <i>Zonotrichia leucophrys</i> | 1                  |
| catbird                                                           | <i>Dumetella carolinensis</i> | 1                  |
| house sparrow                                                     | <i>Passer domesticus</i>      | 7                  |
| blue jay                                                          | <i>Cyanocitta cristata</i>    | 2                  |
| common grackle                                                    | <i>Quiscalus versicolor</i>   | 3                  |
| brown thrasher                                                    | <i>Toxostoma rufum</i>        | 1                  |
| yellow-bellied sapsucker                                          | <i>Sphyrapicus varius</i>     | 4                  |
| downy woodpecker                                                  | <i>Dendrocopus pubescens</i>  | 1                  |
| screech owl                                                       | <i>Otus asio</i>              | 2                  |
| <b>Domestic Birds</b>                                             |                               |                    |
| pigeon                                                            | <i>Columba livia</i>          | 8                  |
| chicken                                                           | <i>Gallus domesticus</i>      | 28                 |
| turkey                                                            | <i>Meleagris gallopavo</i>    | 24                 |
| <b>Mammals</b>                                                    |                               |                    |
| white mouse                                                       |                               | 3                  |
| hamster                                                           |                               | 2                  |
| rabbit                                                            |                               | 2                  |

months<sup>20</sup>. However, it was shown recently that in latent ectromelia of mice, pox virus was isolated from the tail skin of individual mice up to 99 days after infection<sup>10</sup>. In domestic birds with pox infection, the time required for recovery, i.e., for the superficial lesions to dry, form scabs and slough, is four to five weeks<sup>11</sup>. Our own observations on pigeons, however, were that young birds exposed at an age of five days develop extensive lesions that persist up to ten weeks.

Wild birds with avian pox infections have rarely been studied in captivity. During bird banding activities it was possible to observe the pathogenesis of avian pox infection when birds were repeatedly retrapped. Skin lesions persisting up to "several" months were present in chipping sparrows (*Spizella passerina*)<sup>19</sup>. Dunnocks (*Prunella modularis*) with severe lesions on feet and eyes were found to have recovered completely, when retrapped after four months<sup>8</sup>. In pheasants (*Phasianus colchicus*) some of the lesions persisted for two months after their first appearance<sup>5</sup>. Pox lesions on a captive mourning dove (*Zenaidura macroura*) persisted for 82 days<sup>16</sup>. Our own observations on a slate-colored junco (*Junco hyemalis*) were that lesions on a foot disappeared after 81 days in captivity. This is a minimum duration of the infection, however, since this junco was diseased when it was captured.

The long persistence of pox lesions in a yellow-shafted flicker, over a period of 13 months during which intracytoplasmic inclusions were demonstrable, gives rise to some speculation. It appears that some wild birds, probably not only the flicker, are long term carriers of the virus and as such make ideal reservoir hosts. They may play a significant role in dissemination of avian pox infection during migration and also may spread the disease from one parent to another when mating or from parent to offspring during the nesting period. Mosquitoes known as mechanical vectors of pox

virus<sup>3</sup>, have greater opportunity to feed on persistent lesions and thus may contribute further to the spread of the disease. Furthermore, as shown by the partial immunity in one of our flickers, recovered birds can be reinfected. Thus it is ensured that the disease will be maintained for long periods in wild bird populations. We have observed partial immunity in pigeons, as reported by Burnet<sup>4</sup>, 1906, and as observed in chickens by Goodpasutre<sup>11</sup>, 1928. The immunity found in one flicker may have been due to exposure to the virus before capture.

Natural transmission by pecking and fighting is one of the modes of transmission of pox viruses. The injuries in the skin, especially at the angles of the mouth, an area where injury from pecking is common, make an ideal portal of entry for the virus<sup>4 6</sup>.

The fact that 2 flickers developed unilateral keratitis with blindness is noteworthy because it has not been reported in wild birds. In pigeons, keratitis occurred in one or both eyes and it was possible to demonstrate intracytoplasmic inclusions in the affected cornea<sup>4</sup>. Such lesions are found occasionally in chickens<sup>11</sup>.

The failure of attempts to experimentally transmit the virus of the flicker to other domestic and wild bird species or to mammals demonstrates the unique host specificity of the flicker virus. It was impossible even to infect related species of the same family (*Picidae*), such as the downy woodpecker (*Dryobates pubescens*) and the yellow-bellied sapsucker (*Sphyrapicus varius*). Isolation of virus of other wild bird pox strains has been successfully achieved on the chorioallantoic membrane: junco<sup>12 22</sup>; ruffed grouse (*Bonasa umbellus*), sage grouse (*Centrocercus urophasianus*)<sup>7</sup>; brown creeper (*Certhia familiaris*)<sup>15</sup>; song sparrow (*Melospiza melodia*), field sparrow (*Pusilla passerina*) (Kirmse, unpublished). With the flicker virus however, it was not possible to infect 18

other species of birds or to grow it in tissue cultures or on the chorioallantoic membrane of the embryonated chicken egg, even though the persistence of the lesion in the flicker made virus available in abundance and repeated attempts were

made. An alternative explanation may be that the methods used to free the elementary bodies from the inclusion matrix and from the cell were unsuccessful. Further attempts to isolate the flicker virus were hampered by the lack of young flickers or flicker eggs.

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