Isolation of a Herpesvirus from Wild Turkeys in Florida

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SUMMARY
A cell-associated herpesvirus antigenically related and morphologically similar to herpesviruses isolated from domestic turkeys and chickens was isolated in individual kidney cell cultures prepared from four wild turkeys captured in four geographically separated areas in Florida. Serological studies using the indirect hemagglutination test indicated that the virus was widespread in wild turkeys in four widely separated trapping areas in Florida.

INTRODUCTION
Isolation of a cell-associated herpesvirus from domestic chickens, and demonstration of the viral etiology of Marek's disease (1,5,8,13,14,17) have stimulated renewed interest in herpesviruses of other species. A herpesvirus from domestic turkeys which is closely related antigenically to the Marek's disease virus has been used in developing a highly effective vaccine against Marek's disease of domestic chickens (8,11,18). A large proportion of domestic turkeys reared under commercial conditions apparently are asymptomatic carriers of cell-associated herpesviruses. Busch and Williams (3) and Langheinrich (9) have described gross and histological lesions of turkeys which are indistinguishable from the lesions of Marek's disease as seen in chickens. Proof is lacking, however, of an etiological relationship between herpesviruses of turkeys and the Marek's-disease-like lesions. In fact, Witter et al. (19,20)

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observed no evidence of disease during an observation period of one year following inoculation of massive doses of turkey herpesvirus into poults.

This report describes the isolation and study of cell-associated herpesviruses from wild turkeys captured in remote areas in Florida.

**MATERIALS AND METHODS**

**Wild turkeys.** The 46 wild turkeys (*Meleagris gallopavo osceola*) used in this study were captured from four trapping areas in Florida (Fig. 1) between October 30, 1969, and June 16, 1970, by the Florida Game and Fresh Water Fish Commission in connection with research on the biology of the wild turkey population. Turkeys were live-trapped by oral drugs (15) and cannon nets (2), and transported to a laboratory where blood samples were drawn. On the day following capture the turkeys were returned to the trapping area and released. One mature turkey from each trapping area and 15 poults from area 3 were retained for preparation of primary kidney cell cultures. Six fertile eggs were also taken from a wild turkey nest in area 3 and artificially incubated in the laboratory.

**Other wild birds used.** Sandhill cranes (*Grus canadensis*) were present on some of the turkey ranges and sometimes came in to feed at the bait stations. These species were captured in the same manner as turkeys. Primary kidney cell cultures and blood samples were obtained from one sandhill crane, and a blood sample was obtained from one additional sandhill crane. A pen-reared “wild” jungle fowl (*Gallus gallus*) which had been released was captured in area 3, and retained for culture.

**Domestic birds used.** Domestic chickens used were from a flock of single-comb White Leghorns maintained in conventional open housing at the Department of Veterinary Science. The flock had experienced clinical Marek’s disease. Day-old White Leghorn male chicks were obtained from a local hatchery.

Domestic turkeys were obtained from a flock maintained in semi-isolation at the Department of Veterinary Science.

**Virus isolation.** Individual primary kidney cell cultures were prepared essentially as described by Witter (16) from one adult wild turkey captured from each of the four trapping areas; from three 25-day embryos and three one-day-old poults hatched from eggs taken from a wild turkey’s nest; from nine wild-captured five-day-old poults; from six wild-captured five-week-old poults; from
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one adult jungle fowl; from one adult sandhill crane; from four seven-week-old domestic turkeys, and from two seven-week-old White Leghorn pullets. The culture medium employed was lactalbumin hydrolysate 0.25%, yeast extract 0.1%, tryptose phosphate broth 10%, in Earle's balanced salt solution containing 500 units penicillin, 125 μg streptomycin, and 50 units mycostatin per ml. Growth medium contained 5% fetal calf serum which was reduced to 1% in maintenance medium. Cell cultures were incubated at 37°C in an atmosphere of 5% CO₂ and examined daily for cytopathology.

**Virus passage.** Virus passages were in all cases onto primary chick kidney (CK) monolayers. Uninoculated control monolayers were maintained from each passage. Virus passages from infected cell monolayers were attempted by: 1) transfer of supernatant media after centrifugation at 500 × g for 10 minutes; 2) transfer of supernatant media after centrifugation and passage through

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**Fig. 1.** Areas in Florida where the wild turkeys were captured: 1) Lochloosa Wildlife Management Area; 2) St. Cloud; 3) Lykes Fisheating Creek Wildlife Management Area; and 4) Babcock Ranch.
0.45-μ Millipore filters; 3) transfer of intact cells from monolayers which had been removed from culture dishes by the enzymatic action of trypsin; and 4) transfer of cells which had been disrupted by three cycles of freezing and thawing.

**Virus titration.** The amount of virus present in infected monolayers was determined by removal of cells by trypsinization, followed by plating tenfold dilutions of cells on 48-hour CK monolayers essentially as described by Calnek et al. (4). Foci of infection were counted on the fourth day postinoculation.

**May-Grünwald-Giemsa staining.** Forty-eight-hour primary CK monolayers were grown on 22 × 22-mm glass coverslips and inoculated with suspensions of cells from monolayers infected with the

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*Fig. 2. Typical cytopathology produced by a domestic chicken herpesvirus isolate. 350X.*
wild turkey isolate. On the fourth day postinoculation the coverslips were removed, washed twice in Dulbecco's balanced salt solution, fixed for 5 minutes in methanol, and stained by the May-Grünwald-Giemsa technique (7).

**Antigen production.** One virus isolate from a wild turkey, one from a domestic turkey, and one from a domestic chicken were selected for antigen production. The viruses were passaged eight times at four-to-six-day intervals until the cytopathology developed rapidly, and monolayers were extensively involved. Antigens for indirect hemagglutination were prepared from infected monolayers as described by Eidson and Schmittle (6).

**Collection and handling of blood samples.** Blood samples from 46 wild turkeys, one jungle fowl, two sandhill cranes, two domestic turkeys, and two domestic chickens which were survivors of a flock which experienced clinical Marek's disease were obtained by cardiac puncture or venipuncture. Samples were allowed to clot at ambient temperature, and the serums were removed, clarified by centrifugation, and stored at -20 C until used.

**Serology.** Antibody levels were determined by the indirect hemagglutination method as described by Eidson and Schmittle (6). Antibody levels in the serums of 46 wild turkeys were determined by use of the wild turkey antigen. Antibody levels in the serums from two wild turkeys, one jungle fowl, two sandhill cranes, two domestic chickens, one domestic duck, and a pooled sample from fetal calves were determined with all three antigens.

**Electron microscopy.** Viruses were passaged in primary CK cells until cytopathology developed rapidly and the cells of the monolayer were extensively affected. Cells were then removed from the plates by trypsinization and were sedimented by centrifugation, and the resulting pellets were fixed in 3.5% gluteraldehyde, post-fixed in 1% osmic acid, and embedded in Araldite. Thin sections of cells on grids were stained with uranyl acetate and lead citrate prior to examination with a Philips EM 200 electron microscope.

**RESULTS**

**Virus isolations.** Viruses producing cytopathological effects typical of those described for the Marek's-disease-associated herpesvirus were isolated from primary kidney cell cultures prepared from two White Leghorn pullets with gross lesions of Marek's disease. Cytopathological changes were first noted on the fourth day after seeding, consisting of microscopically observable grapelike clusters of rounded, highly refractile cells of fairly uniform size. The round-
Table 1. Serological evidence of antibody against a cell-associated herpesvirus in serum samples collected from wild turkeys.\(^A\)

<table>
<thead>
<tr>
<th>Capture area</th>
<th>Number of serum samples</th>
<th>Reciprocal of indirect hemagglutination titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>512</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>16</td>
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<tr>
<td>4</td>
<td></td>
<td>26</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>46</td>
</tr>
</tbody>
</table>

\(^A\)Antigen was prepared from a wild turkey herpesvirus isolate.

ed cells increased in number, producing large aggregates at each of the original foci (Fig. 2). By the 7th or 8th day the cells in the central portion of many foci detached, leaving a clear area.

Other viruses producing similar cytopathological changes were isolated in primary kidney cell cultures prepared from one adult wild turkey from each of four trapping areas (Fig. 3). Cytopathology, consisting of rounded refractile cells, was first noted in cultured wild turkey cells on the 11th or 12th day after seeding.

Foci of infected cells developed as described for infected chicken cells, but with certain notable differences. The infected turkey cells were more pleomorphic, with many cells becoming greatly enlarged. Individual foci developed to much larger size, becoming grossly observable without magnification.

Only two to six foci of infection developed in the primary kidney cell cultures of each adult wild turkey, suggesting that the amount of virus originally present was quite small.

Other virus isolates producing cytopathological changes identical to those described for wild turkey isolates were made from four clinically normal domestic turkeys. Kidney cell cultures of jungle fowl yielded yet another isolate, which produced cytopathology more closely resembling that of the turkey isolates than of the chicken isolates.

No virus isolations were obtained from wild turkey embryos, one-day-old wild turkey poults, five-day-old wild turkey poults, or the adult Sandhill crane. In no case was cytopathology suggestive of herpesvirus infection seen in uninoculated kidney monolayers prepared from one-day-old Leghorn cockerels.

**Virus passage.** All chicken, turkey, and jungle fowl isolates were found to be highly cell-associated. Infection was readily initiated on primary CK monolayers by passing intact cells from previously infected cell cultures. Infection was not transferred by
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filtered culture media, unfiltered culture media, or cell fragments following disruption by freezing and thawing.

**Virus titration.** Titers of all viruses were increased by serial passage in CK cells. After ten passages, CK cells heavily inoculated with the chicken isolate were producing approximately $7 \times 10^4$ plaqueforming units (PFU) of virus per 60-mm petri plate. Chicken kidney monolayers similarly inoculated with tenth-passage wild or domestic turkey virus isolates produced approximately $3 \times 10^5$ PFU of virus per plate.

**May-Grünwald-Giemsa staining.** The enlarged rounded cells comprising the microplaques produced by the wild turkey isolate stained intensely with May-Grünwald-Giemsa. Cowdry type A inclusions were present within the nuclei of some infected cells.

**Serological studies.** Table 1 presents serological evidence of

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*Fig. 3. Typical cytopathology produced by a wild turkey herpesvirus isolate. 350×.*
Table 2. Serological relationships of wild turkey, domestic turkey, and domestic chicken herpesviruses as measured by indirect hemagglutination.

<table>
<thead>
<tr>
<th>Antigens prepared from</th>
<th>Wild turkey herpesvirus</th>
<th>Domestic turkey herpesvirus</th>
<th>Domestic chicken herpesvirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild turkey</td>
<td>512&lt;sup&gt;A&lt;/sup&gt;</td>
<td>512</td>
<td>64</td>
</tr>
<tr>
<td>Wild turkey</td>
<td>128</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>Domestic turkey</td>
<td>8</td>
<td>8</td>
<td>Neg.</td>
</tr>
<tr>
<td>Domestic turkey</td>
<td>16</td>
<td>8</td>
<td>Neg.</td>
</tr>
<tr>
<td>Domestic chicken</td>
<td>512</td>
<td>512</td>
<td>512</td>
</tr>
<tr>
<td>Domestic chicken</td>
<td>256</td>
<td>128</td>
<td>16</td>
</tr>
<tr>
<td>Jungle fowl</td>
<td>8</td>
<td>4</td>
<td>Neg.</td>
</tr>
</tbody>
</table>

<sup>A</sup>Reciprocal of serum dilutions.

widespread exposure to the cell-associated herpesvirus in the wild turkey populations of the various study areas. Antibody titers ranged from negative to 1:512, the highest dilution routinely used. Indirect hemagglutination titers of the turkeys from which virus isolations were made were: area 1, negative; area 2, 1:64; area 3, 1:8; and area 4, negative. Three five-day-old poults known to be from the same brood had antibody titers of 1:512. Six other five-day-old poults from two other broods had no measurable antibodies. Antibody titers of eight five-week-old poults ranged from negative to 1:32.

There was evidence of many cross reactions among the three viruses studied (Table 2). Serum samples producing hemagglutination at a dilution of 1:64 or higher with any given antigen also reacted at a dilution of 1:8 or higher with the other test antigens.

**Electron microscopy.** Intranuclear herpesvirus particles present in infected cultured cells from domestic chickens, domestic turkeys, and wild turkeys were morphologically similar. Virions were 98 to 102 \( \mu \) m in diameter, and some contained a viral core in the shape of a cross. Small particles, 50 to 60 \( \mu \) m in diameter, were also present in the nucleus. Fig. 4 is an isolate from a wild turkey. The jungle fowl isolate was not examined by electron microscopy.

**DISCUSSION**

Virus isolates described in this study were classified as members of the herpesvirus group for the following reasons:

1) Cytopathological changes typical of herpesvirus were produced in cell culture.

2) The cell-associated nature of the isolates was suggestive of group B herpesvirus.
3) Cowdry type-A intranuclear inclusions were demonstrated by May-Grünwald-Giemsa staining.

4) The intranuclear location, size, and shape of the virus particles as demonstrated by electron microscopy were compatible with those described for herpesvirus of chickens and turkeys (10).

5) The small intranuclear particles often associated with herpesviruses of chickens and turkeys were present.

6) Many cells infected with the turkey isolates displayed the "electron-lucent cross" configuration described for domestic turkey herpesviruses.

The discovery that a turkey herpesvirus could be used as a vaccine for preventing Marek's disease in chickens, and the phenomenal success of such vaccines in the field, have raised questions concerning the origin of the cell-associated herpesviruses of domestic turkeys, the length of time that such agents have been present in turkeys, and how widespread the infection is in turkey populations. The isolation of herpesviruses antigenically related to domestic turkey and chicken isolates from four of four wild turkeys, and sero-
logical evidence of exposure in four distinctly separated wild turkey populations, suggest that the virus is quite widespread and is probably not a recent event in turkeys.

The cell-associated virus was apparently able to persist for long periods at a very low level in turkeys without clinical effects or stimulation of high antibody levels. Poults may be hatched with a high or low level of parental antibody, presumably reflecting the antibody level of their dam.

Failure to isolate virus from wild turkey embryos, newly hatched poults, five-day-old poults, and five-week-old poults suggests that the virus is probably not egg-transmitted. This observation is in agreement with findings of Prem et al. (12) in studies of domestic turkey herpesviruses, and results of Solomon et al. (14) with Marek’s disease virus of chickens. Additional evidence that chicken herpesviruses are not egg-transmitted is provided by the absence of herpesvirus in pooled samples of kidney cells from one-day-old chickens which were processed and grown as cell cultures weekly for almost a year.

The antigens prepared from wild and domestic turkey isolates which were used for serological comparison were probably of higher titer than the chicken herpesvirus antigen. Even though all antigens were produced and handled in an identical manner, the turkey isolates produced cytopathology more rapidly and the infection progressed until higher proportions of cells were infected than was possible with the chicken isolate. This presumably resulted in more virus present in the cells from which the turkey antigens were prepared.

REFERENCES


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