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ISOLATION AND CHARACTERIZATION OF A HERPESVIRUS FROM WILD TURKEYS (*Meleagris gallopavo osceola*) IN FLORIDA[1] [2] [3]

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Abstract: Viral agents producing both a syncytial-type cytopathic effect and type A intranuclear inclusion bodies *in vitro* were isolated from the kidneys of five of 10 wild turkeys. A plaque assay system for viral infectivity was developed and used to characterize one of the wild turkey viruses (WTV). WTV replication was inhibited by 5-bromodeoxyuridine, indicating the virus contained DNA as its genetic material. Virus infectivity could be transferred only as viable whole cell preparations; one cycle of rapid freezing and thawing completely inactivated the virus. Typical herpes-like virions were found within the nuclei when cells infected with WTV were examined by electron microscopy. WTV had characteristics typical of the herpes group of viruses.

INTRODUCTION

In 1971 Busch and Williams[1] described neural and visceral lesions in wild turkeys resembling lesions seen in chickens with Marek's disease. A virus possessing some characteristics of the herpes group was later isolated from apparently normal wild turkeys.[2] Based on previous reports of a putative herpesvirus in wild turkeys, our objectives were: (1) attempt to confirm the reported isolation of a herpesvirus from wild turkeys in Florida,[2] (2) develop a plaque assay system for the virus, and (3) determine some of its characteristics.

MATERIALS AND METHODS

Virus isolation and propagation

Virus isolation was attempted on nine apparently normal, juvenile, male wild turkeys (*Meleagris gallopavo osceola*) and on one 5-year-old adult male, all captured in Lykes Fisheating Creek Wildlife Refuge in South Florida by personnel of the Florida Game and Fresh Water Fish Commission. Kidneys were removed aseptically and cells prepared for seeding as described by Kawamura *et al.*[5] Cells were grown in 0.5 or 1 l prescription bottles with a growth medium consisting of a mixture of Ham's F-10 and medium 199.[5] After 7 to 12 days monolayers were subcultured by dispersing cells with 0.025% trypsin. New cultures were seeded at a ratio of 1:2.

Stock virus was prepared by seeding wild turkey kidney cells with advanced cytopathic effects (CPE) into monolayer cultures of duck embryo fibroblast (DEF) cells. DEF cells were prepared

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as described by Solomon *et al.*[8] and maintained with the growth medium described above. After the development of CPE in DEF cultures, monolayers were dispersed with trypsin solution and the cells resuspended in growth medium containing 7% dimethyl sulfoxide. Infected cells were preserved by freezing[6] and held at —90 C.

Virus assay

Monolayers of DEF cells were grown in 60 mm plastic tissue culture dishes (Falcon Plastics, Oxnard, California) at 37 C in a humidified atmosphere containing 5% CO_2. After 24 h the growth medium was removed and 1 ml each of serial five-fold dilutions of virus was inoculated in triplicate into the cultures. Small plaques, which formed within 6 to 7 days, were counted with the aid of a microscope and a 7 cm grid containing 2 mm squares (Technical Instrument Co., San Francisco, California) or by direct plaque count after the cell sheets were stained with 1% crystal violet. A linear dose-response curve was obtained when the number of plaques formed was plotted as a function of dilution.

Electron microscopy

For electron microscopic studies, monolayers of infected DEF cells with extensive CPE were dispersed with trypsin and the cells firmly pelleted by centrifugation. The supernatant fluid was discarded, the cells fixed in 3.5% buffered glutaraldehyde, post-fixed in 1% OsO_4, and embedded in Araldite. Thin sections of cells on grids were stained with lead citrate[7] prior to examination with a Philips EM200 electron microscope.

Nucleic acid determination

The nucleic acid type was determined indirectly by use of 5-bromodeoxyuridine (BUdR). DEF monolayers in tissue culture dishes were inoculated with 1 ml of serial five-fold dilutions of viral suspensions. Four ml of medium containing 0.1 mg BUdR/ml were added to the infected cultures and the reduction in formation of plaques was determined after 4 and 7 days. As a control, the effect of BUdR on Newcastle disease virus (an RNA-containing virus) was also determined.

RESULTS

Development of CPE

Cultures of wild turkey kidney cells were confluent after 24-48 h of incubation, and the first signs of degenerative changes in cell sheets were evident 6 to 7 days after transferring the original cultures. Early CPE was characterized by the presence of groups of cells which were more refractile than adjacent cells. As CPE progressed, increasing numbers of cells became more refractile and large syncytia formed. Infected cells were then transferred to cultures of DEF. Similar cytopathic changes were detected in kidney cell cultures from five of the 10 turkeys. One of the isolates was selected for characterization.

After infected cells were stained with hematoxylin and eosin, examination by light microscopy revealed large cells containing numerous intranuclear bodies. These inclusion bodies are shown in Figure 1 and are the type A described by Cowdry.[3] Upon examination by electron microscopy, infected DEF cells were found to contain viral particles within the nuclei. Viruses were not found in uninfected DEF cells. Virions averaged 129 nm in diameter, and some contained cores about 82 nm in diameter (Figure 2). These cores were shaped like a maltese cross.

Nucleic acid type

A definitive determination of the type nucleic acid contained in the viral genome is usually based on isolation, purification, and physical characterization of the nucleic acid. However, in some cases this rigorous proof is not feasible because of problems involved in purification of the virus and, thus, susceptibility

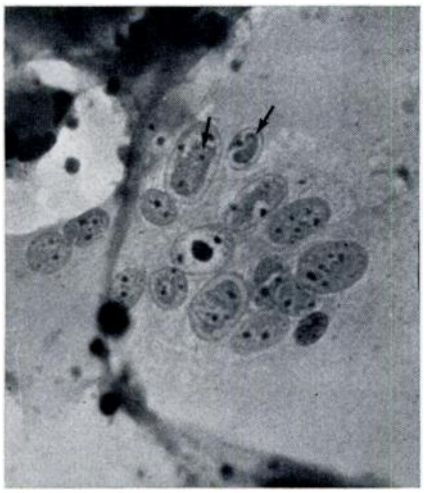

FIGURE 1. DEF cells infected with WTV. Type A intranuclear inclusion bodies are shown after cells were stained with hematoxylin and eosin (arrows). 438X.

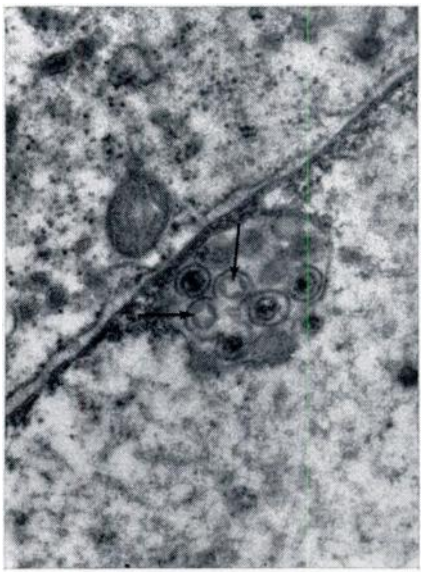

FIGURE 2. Electron micrograph of naked WTV virions in the nucleus, some of which have a viral core in the shape of a maltese cross (arrow). 55,000X.

to deoxyuridine inhibition of viral replication has been used to indicate the type of nucleic acid present in the virion.[4,5] When DEF cells were infected with WTV and then treated with BUdR, plaques did not form (Table 1), indicating that replication of WTV was inhibited and that the virus probably contained DNA. Plaque formation by NDV, an RNA-containing virus, was not inhibited under similar conditions.

Stability

WTV was completely inactivated by one cycle of rapid freezing and thawing. Furthermore, infectivity could be preserved by freezing only as viable whole cell preparations. Infectivity could not be transferred with cell-free medium from infected cell cultures.

TABLE 1. Effect of BUdR on plaque formation by WTV.

	Viral titer[a]	
Treatment	WTV pfu/ml	NDV pfu/ml
Control	2.24×10^4	9.08×10^5
BUdR	0	1.05×10^6

[a] average of 3 separate determinations

DISCUSSION

Data presented here indicate that the virus isolated from wild turkeys contains DNA as its genetic material, as was demonstrated when plaque formation by WTV was inhibited by BUdR. Kawamura *et al.*[5] and Izawa *et al.*[4] reported that the herpesvirus of domestic turkeys contains DNA because of its sensitivity to BUdR. Site of replication is apparently the nucleus as indicated by the formation of intranuclear inclusion bodies and presence of naked virions in the nuclei of infected cells. These virions were morphologically similar to those seen in cells infected with the herpes-like virus isolated from wild turkeys by Colwell *et al.*[2]

Our study confirmed the report by Colwell *et al.*[2] of a herpesvirus in wild turkeys. We isolated the virus from 50% of the birds cultured. Whereas they propagated the isolate in primary kidney cells from chicks that were apparently not from Marek's disease-free flocks, we circumvented the problem of contamination with Marek's disease virus by using cultures of duck embryo fibroblast cells. Further studies of this agent should include serologic studies to establish the antigenic similarity to other known herpesviruses and pathogenic studies to determine if the agent causes clinical signs of disease in young wild turkey poults.

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