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Isolation of Pox Virus from Peacocks (*Pavo cristatus*) in Mosul

[1]M.Y. AL-Attar, [1]S.Y. AL-Baroodi and [2]S. Muneer Al-Badrany
[1]Department of Microbiology, [2]Department of Surgery and Obstetrics,
College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract: Avian pox virus was isolated from skin pox lesions and oropharynex region swabs taken from peacocks (*Pavo cristatus*). The isolated virus produced small haemorrhagic plaques on Chorioallantoic Membrane (CAM) of developing chicken embryos. The isolated virus diagnosed by agar gel diffusion test and serum neutralization test. Morphological identification using negative staining technique of wet preparation of isolated virus is conducted and examined under the electron microscope showed oval to brick shaped particules; their sizes ranged from 300-350 × 150-230 nm. Chickens inoculated with the virus by scarification developed localized pox-like lesions but turkeys showed cutaneous lesions on head, legs and transient swelling of feather follicules at the site of inoculation, where as pigeon showed no lesions.

Key words: Avian pox, virus, peacock, isolation, electron microscope

INTRODUCTION

Avian pox virus infection have been described since the middle of the 19th century as infectious disease caused by a family of viruses collectively known as avipox viruses which are antigenically and immunologically distinguishable from each other, but cross relationships complicate strain identification (Tripathy and Reed, 2003).

Serology has revealed cross-reactivity among several of the viral species, this disease affects more than 232 species of 23 orders of birds (Murphy *et al*., 1995) including fowl, turkey, pigeon, canary, quail, sparrow and starling (Bolte *et al*., 1999).

In spite of wide range of species of birds and strains of the virus the associated pathology is very similar, the most common cutaneous form of avian pox involves the unfeathered parts of the body, face, eyelids, base of the beak and legs. Lesions consist of epithelial hyperplasia of the epidermis, but with the diphtheritic form, caseous, necrotic lesions develop in the mucous membranes of the upper respiratory tract, mouth and pharynx (Tripathy and Reed, 2003).

In Iraq, Peacocks (*Pavo cristatus*) are reared in some farms or houses as pets. The first peacock pox infection was reported in Baghdad Zoo (AL-Falluji *et al*., 1979).

In this study we attempted isolation of pox virus from naturally infected peacocks in Mosul, as well as studying of morphological and host specificity characters of this virus.

MATERIALS AND METHODS

Samples: Skin lesions in the upper site of the head, eyes and dark brown nodules in the hind toe, which were collected from naturally infected peacocks (Fig. 1). and swab samples were taken from oropharynx region (Fig. 2). All lesions were preserved in phosphate buffer saline mixed with glycerin pH = 7.2 containing 100I.U mL^{-1} penicillin and 100 mg streptomycin and kept at -20°C till virus processing (Rai, 2005).

Virus isolation: The collected samples were prepared as described earlier (Cox, 1980). The virus suspension was inoculated on the Chorioallantoic Membrane (CAM) of 11-12 days old of local breed chicken embryos, then incubated for 7 days at 37°C with daily examination.

Membrane with pock lesions were collected for further passages of the virus. Five successive passages of the virus were carried out and the titers were calculated (Reed and Muench, 1938).

Virus identification

Serological identification

Agar gel diffusion test: Harvested (CAM) isolated virus was used against convalscent serum collected from infected peacocks and hyperimmune serum was used

Corressponding Author: M.Y. AL-Attar, Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

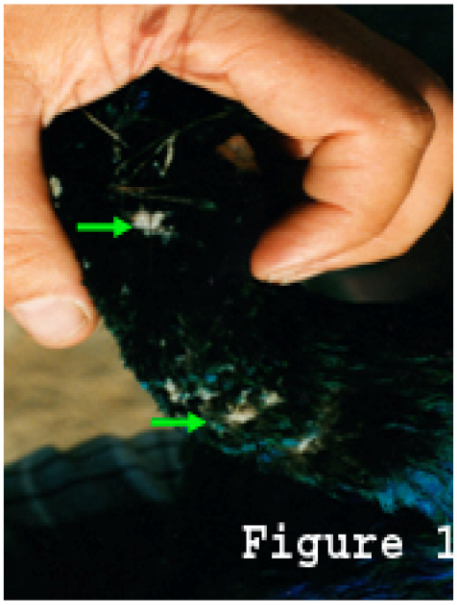

Fig. 1: Cutaneous lesion in the head

Fig. 2: Diphtheric lesion in oropharynex region

against fowl and pigeon pox using 1% agarose in distilled water, then incubated for 48 hours in humid chamber at room temperature (Yavuz *et al*., 2005).

Serum neutralization test: Two fold dilution were done for convalescent serum of infected peacock, hyperimmune serum against fowl and pigeon pox also used, then 100EID_{50}/0.1 mL of isolated virus was mixed with equal volume of each serum, the mixture was kept for 1 h at 37°C, 0.1 mL of each dilution were inoculated on CAM, the result was calculated as described before (Smits *et al*., 2005).

Morphological identification: Negative staining technique of wet preparation of isolated virus was utilized using Phosphotungistic Acid (PTA) stain and examined under the electron microscope at AL-Nahrin University-Baghdad (Tajima and Ushijima, 1966).

Host specificity

The following three types of birds were used in this study

Chickens: six weeks old chickens were intravenously inoculated with 0.2 mL ($2\times10^{5.7}$ EID_{50}/0.1 mL) and skin scarification (Minbay *et al*., 1973).

Pigeon: About six months old pigeons were inoculated using the same method and dose of isolated virus like that done in chickens (Bolte *et al*., 1999).

Turkeys: Four months old turkeys were intravenously inoculated using feather follicules method (Ideris and Ibrahim, 1986).

RESULTS

Virus isolation: The virus isolated from both the skin lesions and oropharynex swabs on CAM produced small haemorrhagic plaques 5 days after inocualtion; the plaque gradually increased to 2-3 mm in diameter (Fig. 3).

After the fourth and fifth passages and the titer of isolated virus increased gradually to reach $2\times10^{5.7}$ EID_{50}/0.1 mL of fifth passage and causing embryo death. (Table 1).

Serological identification

Agar gel diffusion test: Isolated virus showed clear precipitation line with convalescent serum only.

Serum neutralization test: The convalescent serum neutralized the isolated virus at titer of 8.

Morphological identification: Under the electron microscope, the virus was abundant, oval and brick shaped; its size about 300-350×150-230 nm at 34000X (Fig. 4)

Host specificity

Chickens: Localized cutenous lesions were observed on inoculated chickens by skin scarification route with 40% infectivity rate and 20% mortality.

Table 1: Titer of isolates of each passage and the lesions in the embryo and CAM

Type of lesion	No of passage	Titer of virus EID50/0.1 mL	Mean Death time hrs	Congestion in CAM	Pock lesion in CAM	Edema in CAM
Cutenous lesion	P1	10^{2}	--	+	--	+
	P2	$10^{2.9}$	--	+	--	+
	P3	$10^{3.7}$	120	++	--	++
	P4	$10^{4.5}$	96	+++	+	+++
	P5	$10^{5.1}$	96	+++	++	+++
Diphtheric lesion	P1	$10^{2.5}$	--	+	--	+
	P2	$10^{3.3}$	120	++	--	++
	P3	$10^{3.9}$	96	+++	+	+++
	P4	$10^{4.8}$	72	+++	++	+++
	P5	$10^{5.7}$	72	++++	+++	++++

(--) no changes, (+) mild congestion , (++) moderate congestion
(+++) Acute inflammation, (++++)sever inflammation

Table 2: Results of experimental infection of virus isolated from peacocks in different birds

Bird spp	No. of birds	Route of inoculation	Cutenous lesion	Diphtheric lesion	Infectivity rate %	Mortality rate %
Turkey	3	IV	+++	+++	100	66
	3	IF	+++	+	66	33
Pigeon	20	Scarification	--	--	0	0
	20	IV	--	--	0	0
Chicken	20	Scarification	+	--	40	20
	20	IV	--	--	0	0

+++ Acute lesion + Mild lesion--No. lesion appeared
IV Intravenous IF Intrafollicular

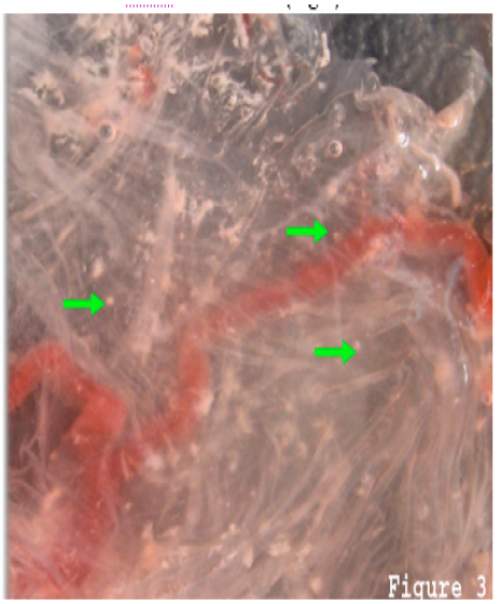

Fig. 3: Pock lesions and congestion of chorioallantoic membrane

Fig. 4: Virus particles under electron microscope (34000X)

Pigeon: Showed no lesions in two routes of inoculation.

Turkeys: cutenous lesions were noticed of head, legs and transient swelling of feather follicules at the site of inoculation, in both IV and IF routes of inoculation. Diphtheric form lesions were noticed in IV inoculation more than IF route (Table 2).

DISCUSSION

The work has shown that the pox virus isolated from peacocks is related to Avian pox virus group and is highly pathogenic to chickens and turkeys. This is different from that reported by AL-Falluji *et al.* (1979), who found that peacock pox virus is highly pathogenic only to chickens but not to turkeys.

The diagnosis which is considered the first in Iraq, for such types of viruses, is supported by the demonstration of pox virus particules using electron microscopy, the varying shapes of the virus particles probably represent different development stages as described earlier (AL-Hyali and AL-Jumily, 2002).

Direct electron microscopy of negatively stained preparations provides a reliable and rapid genus diagnosis but no species diagnosis of pox viruses (Boulanger *et al*., 2002).

CONCLUSION

Avian pox virus can be isolated from peacock birds on chorioallantoic membrane of local breed chicken embryo, the isolated virus is highly pathogenic to chickens and turkeys. Electron microscope positively supports the serological diagnosis of the isolated virus.

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