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Virological Study about *Avipox virus* in Local Birds of Iraq

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Abstract: ***Background: Avipox virus considered to be a contagious disease of many types of birds wild and domestic. The current study focused on the isolation of recently common Avipox virus which include fowl pox (FP) and pigeon pox (PP) viruses in Iraq. Materials & methods: Isolation of the viruses was done via chorioallantoic membrane (CAM) route of avian embryo inoculation, also used by a chicken fibroblast cell culture of adapting virus to detect of cytopathic effect (CPE). Clinical manifestations of apparently infected birds, which showed a typical "pox lesion" that appeared in different parts of the face, only cutaneous susceptible parts were interested in thecurrent study. Results: The isolated viruses were studied which leads to a similar features in the membrane (CAM). Pocks lesion were produced by these isolates approximately 5 mm diameter or less at the (3rd) third and (4th) fourth passage, at the first passages thickening and necrosis of CAM were shown only . "Intracytoplasmic inclusion bodies are found on histopathology of CAM and at the infected site. At third and fourth passage concerning cell culture, the chicken embryo fibroblast (CEF) inoculated with the virus and showed the characteristic cytopathic effect (CPE) which included aggregation of cells and detachment of the cells from monolayer". Conclusion: The main observations were shown which express the typical manifestation of pox Viridae on cell culture and embryonated chicken egg.***

Keywords: Fowl Poxvirus, Pigeon Poxvirus, cell culture, embryonated chicken egg

1. Introduction

"Poxviruses (*poc, pocc, mean* pustule) virions are brick shaped and complex in symmetry, ranging about 250 x 200 x 200 nm in size [1]. The genome consists of a single linear molecule of double-stranded DNA, Fowl pox is a contagious disease of domestic and wild birds [1] of all ages, sexes and breeds [2] which is caused by fowl pox virus (FPV), DNA virus belong the genus *Avipoxvirus* of family *Poxviridae* and subfamily *Chordopoxvirinae"* [3].

"Replication cycle occur within the cytoplasm (called *viroplasm* or *viral factories),* and the mature virion were released by budding (enveloped virions) [1, 6]. The virus can transmitted by direct contact (including wounds, abrasions), or by fomites, aerosol, or mechanically by arthropods (insects) [4, 7] and wild birds [8]. Genus *Avipoxvirus* which include many specific bird poxviruses, including fowl pox virus, canary pox virus, and pigeon pox virus"[5].

Apparently affected birds were showed three forms of the disease called ; the skin form" (cutaneous), diphtheritic and systemic forms [9, 10]". In the cutaneous form, birds have nodular lesions on unfeathered parts of the body [1]. The characteristics picture of a diphtheritic form is bluish fibronecrotic lesions in the mucous lining of the throat (oropharengeal) [2], while in the third form the visceral organs were obviously influenced [9]. Major interest was prerequisite because this virus infection accompanied with high frugal loss. Mortality rate was increased when the disease was accompanied by secondary bacterial infection [11, 12].

"For the appropriate investigation and diagnosis, viruses are isolated either in cell culture (chicken fibroblast), or embryonated chicken eggs using CAM route or by the combination of both techniques [13, 14]. Fowl pox is an emerging disease [15] and the variant FPV has been reported broadly" [16]. In the current study was concentrated on isolation of *AVIPOX* virus from different places of Wasit and Baghdad provinces .

2. Materials and Methods

Specimens collection

The study was conducted between March 2016 and September 2016, fowl pox lesions were collected from affected birds. Clinical features of affected birds were characterized by the presence of crusts or nodular lesions showed on the face ("comb, wattles, eyelid, legs and other unfeathered body part)".

Scabs (nodular lesions) were collected under aseptic condition to reduce contamination from (70) local chickens and pigeons have clinical symptoms (40 chickens and 30 pigeons) gathered from private bird show places. These samples were kept in screw tied vials and stored at 4°C. Laboratory investigations were done in the department of Veterinary centers of Baghdad.

Preparation of specimens and isolation of the virus

"Scab collected from infected birds were minced separately in sterilized equipments include mortar and pestle and suspended in sterilized phosphate buffered saline (PBS) to make 10% suspension. The suspension was centrifuged at 3000 RPM for 15 min and the supernatant was treated with a mixture of Penicillin and streptomycin for 45 min at 37°C "to reduce bacterial contamination, in addition to Antifungal drug (Nystatin).

"All the field virus stock (0.5 ml) sample was initially inoculated on to the CAM of embryonated chicken egg at 12 days old as mentioned in [17]. After the inoculation, incubated at 37°C were done and checked every day. At the

5-6 days post inoculation, (PI) CAM was collected and examined for pock lesion. Subsequent passages were required for further adaptation of the virus in CAM".

Cell culture at embryonated chicken fibroblast

"Several subsequent passaging of one sample of field strain of fowl pox was done in chicken embryo fibroblast cell culture. The primary fibroblast cell culture was prepared from 10-13 days old chicken embryos, according to the method used by [18], by using ready formulated media (Eagle). Uniform suspensions of cells were obtained by the trypsin digest method [19]. The uniform cell sheets (monolayer) were formed after 48-72 hours of incubation".

Virus isolation in cell culture (chicken fibroblast)

"The virus samples were inoculated in the CEF cell culture. The monolayers in special falcons were inoculated with 0.5 ml of the virus (adapted on CAM). After 1.5 hours of virus adsorption at 37°C the extra amount inoculum from falcons were discarded and maintenance medium was added and incubated at 37°C. After 3 and 5 and 7 and 10 days PI, the medium from inoculated falcons were collected after three cycles of alternate freezing and thawing. A part of the collected samples were inoculated in to a fresh of another five cell culture falcons at 0.2 ml each one while an original one was preserved at −70°C "[20].

Histopathology of inoculated CAM and affected parts

"To detect the virus and pathological findings, pocks on the CAMs were subjected to histological examination, in addition to affected parts . The CAM showing lesions were fixed at 10% buffered formalin solution.

The fixed CAMs were embedded in paraffin and cut it to 6-8 µm thick. Sections were stained with hematoxylin and eosin and examined microscopically for the presence of cytoplasmic inclusion bodies"[21] .

Results &Discussion

Clinical features of infected birds

The live chickens and pigeons showed the typical pox lesions, which include nodules and cruststs on the unfeathered skin such as comb and wattles in addition to eyelids this may lead to conjunctivitis or hemorrhagic ulcerations with secondary complication in eyes as shown in figure (1).

These results were in line of [1, 22]who showed same gross pathological affections in the chickens and pigeons, also attributed it to poor physiological and environmental condition, such as hot weather and presence of insect.

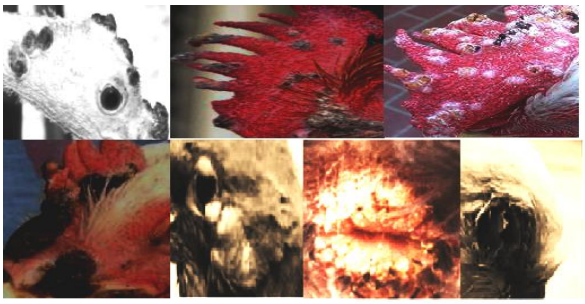


Figure (1): The cutanous clinical form of infection in chicken and pigeon ,include nodular and scabs at unfeathered skin of head and cloaca with some complications

Inoculation of virus stock in to embryonated chicken egg (ECE).

The virus stock was prepared under strict aseptic condition to reduce contamination. The virus was propagated at the Chorioallantoic membrane (CAM) of the embryo at the age 10-13 days with special technique . Grossly, the pock lesion that appeared at the CAM with the 1st passage which include, thickening, dull, may be edematous as shown in figure (2, A). Progressively, when the virus was adapted well, which was leading to appearance of round raised necrotizing points reach to 5 mm diameter at 3rd passage as in figure (2, B). It was clear from figure (2, C) which showed congested hemorrhagic lesions at 4th passage .

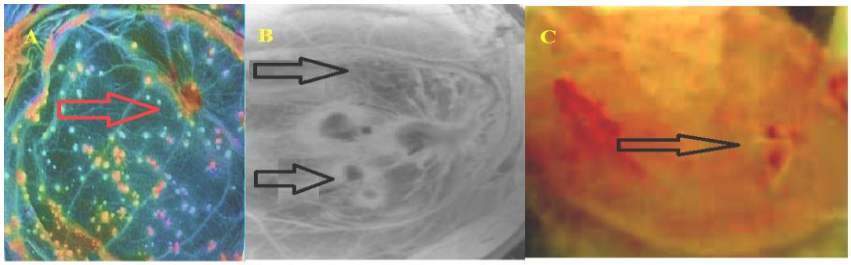


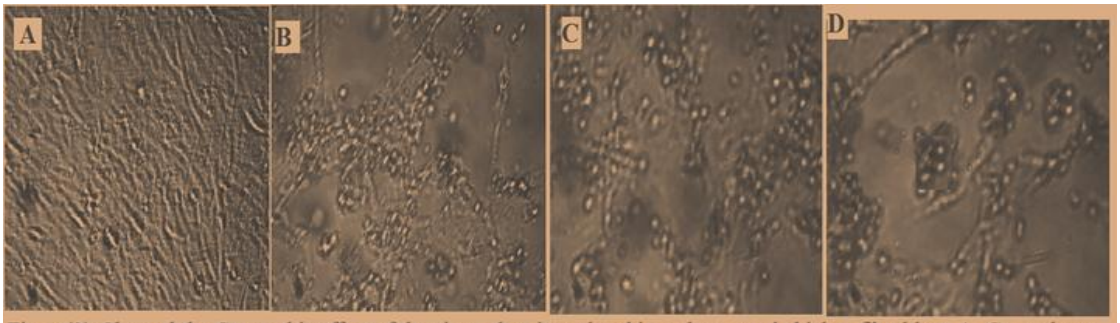
Figure (2): Showed the pathological findings on CAM . A. Thickening of CAM at 1st passage . B. Pock lesion of CAM at 3rd passage . C. Apparent pock lesion (hemorrhagic) at 4th passage of the virus (fowl pox).

The current observations considered to be compatible with the results of [1, 23] who showed that the virus had a tendency to grow on the CAM and produce the characteristic lesion which was shown.

Isolation of the virus using of embryonated chicken fibroblast (ECF)

This type of the cells considered to be good for *AVIPOX* multiplication as suitable host system [23]. The adapted CAM virus was inoculated on ECF and thus showed the cytopathic effect (CPE). Clearly, the 1st and 2nd passage were showed no obvious effect, but the effect was started at 3rd passage that showed aggregation and some cells floatation at the surface of the media.

At 4th passage, the signs were appearing more severe which include rounding as grape clusters with the signs of degenerative changes specially at 24 hr. (P.I), whereas the signs become more severe with excessive cell detachment from monolayer which showed at 48 hr. P.I in addition to previous effects as shown in figure (3. A, B, C, D). [23].



Figure(3): Showed the Cytopathic effect of the virus when inoculated in embryonated chicken fibroblast. A- Control non infected cell culture. B- Cell aggregation and show sloughing of it at 3rd passage. C & D. Showed rounding and detachment of aggregated cells from monolayer after 24 and 48 hr. P.I of 4th passage respectively (40X).

The current results were supported with the observation of [23, 24, 25] who showed exactly the same effects of the virus on this type of the cells .

Histopathological findings of affected parts and CAM

At necropsy, the histopathological effect of the virus on the susceptible cutaneous tissues which revealed presence of hyperplasia of epidermal cells, also there was ballooning degeneration of skin epithelial cells (stratified squamous), the epithelial cells were shown separated and swollen so as to rounded at stratum spinosum layer.

Furthermore, there were special eosinophilic inclusion bodies which was called (Bollinger bodies), there's neutrophils and mononuclear cells, which infiltrated at the dermis, as shown in figure (4. A, B, C). The section of infected (CAM) was the presence of large intracytoplasmic inclusion bodies that it was pathognomic for *AVIPOX* infection as in figure (4.D). These outcomes were cooperated with the investigation of [22, 23] who showed the same results of infected tissues.

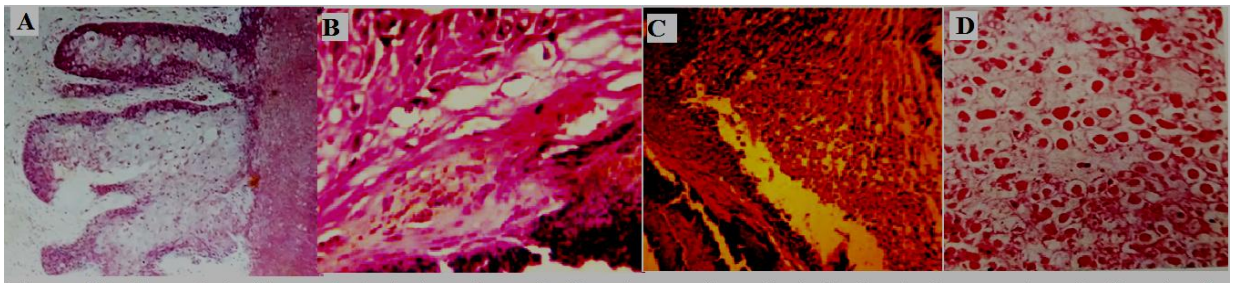


Figure (4): Showed the histopathological sections of affected parts showed A. Hydropic degeneration of affected cells and ballooning and showed of likely intracytoplasmic inclusion bodies with proliferation of superficial epithelial cells . B. The hydropic degeneration of prickle cell layer .C. Large number of dead neutrophils on the surface with mononuclear cells infiltration in the dermis in addition to acanthosis and hyper keratosis at the epidermis. D. Section of CAM showed large number of eosinophilic intracytoplasmic inclusion bodies(H & E stain 40X).

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