Isolation of Pigeonpox Virus from Severe Infection of Pigeons in Diyala province: Virological and Histopathological Study

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Summary

Pigeonpox virus was isolated from severe cases of avipoxvirus infection affecting 64 pigeons in Ba'aquba of Diyala governarate. The virus grew well on chorioallantoic membrane of chick embryos of 11-12 days old, and produced typical pock lesions. Histopathological sections of infected tissue samples revealed typical pox lesions. The virus was identified as Pigeonpox virus using of specific hyper immune serum and indirect immuno-flourescent and indirect immuno-peroxidase tests. The virus agglutinated RBCs of pigeon, fowl, turkey and duck. Experimental infection in pigeons produced moderate infection as compared to the diseased birds, while in chicken the virus produced mild infection.

Keywords: Pigeonpox, Avipoxvirus, Indirect immunoperoxidase, Indirect immunoflourescent.

Introduction

Pigeon pox virus is a member in *Avipoxvirus* genus that belongs to subfamily *Chordopoxvirinae* of the family *Poxviridae* (1). Other members of *Avipoxvirus* and pigeon poxvirus are closely related to each other and are not species specific (2 and 3). Pigeon pox virus can be used as a vaccine against fowl and pigeon poxvirus infection and also against other avian poxviruses (4 and 5). Pigeonpox virus infection is worldwide in its distribution (6-8). In Iraq, the virus was reported longtime ago when it was isolated and compared to fowl pox virus in both characteristics and experimental infection (9). In Baaquba of Diyala governarate, the virus has been reported clinically in many locations of pigeons but it was not isolated and identified or compared to other avian-poxviruses. Accordingly, the aim of this study is to isolate the virus and study of some of its characteristic from virological and histopathological points of view.

Materials and Methods

Clinically severe cases of pigeon poxvirus infection were admitted to the private Veterinary clinic in Baaquba city of Diyala. The head and especially the area around peak were heavily covered with lesion. Also the infection appeared heavily in the vent area around cloaca, especially in dead birds it was shown the same lesions. Papules, pustules, scabs and skin samples were collected from both peak and vent areas. Some samples were kept at -20 °C until use and the others were kept at 10% buffered formalin for histopathological and histo-immunological tests. Collected samples were processed as described by (10). Embryonated hen eggs 11-12 days old obtained from local hatcharies were used for virus isolation and 0.1 ml of processed sample was inoculated on chorioallantoic membrane (CAM) (11). The inoculated eggs were incubated at 37 °C and observed daily for seven days. The inoculated CAM of embryonated eggs was collected and checked for pock lesions or any other changes. CAMs that showed pock lesions were processed to 2nd, 3rd, 4th and 5th passage on CAM. CAMs with clear pock lesions were kept at 10% buffered formalin and processed for histo-immunological tests.

Collected samples from skin, papules and pustules were kept in 10% buffered formalin, paraffin embedded and processed for histo-pathological examination (12). Tissue samples that processed and embedded in paraffin were cut into 5-10 and stained by Hematoxilin and Eosin stains (13). The third, fourth and fifth passages of the virus on CAM were titrated. The pock forming units were calculated according to number of pock formed on CAM of highest dilution (11). To determine the ability of the virus to agglutinate avian RBCs, slide heamagglutination test was performed with 5% RBCs collected from fowl, turkey and ducks. Five microns thickness paraffin embedded skin and CAM tissue samples were

used in indirect immuno flourescent (IIF) test. The paraffin embedded sections of infected samples were subjected to dewaxing in two changes of xylene four minutes each. Furthermore, they were processed for IIF (14 and 15) by the use of FITC conjugated rabbit anti chicken IgG (Nordek, Buckingham, UK) and anti-pigeonpox virus hyperimmune serum. For test the same above-mentioned procedure was followed except that the secondary antibody was conjugated with horse-radish peroxidase instead of FITC, and 4-Chloro 1-Napthol was used as a substrate. For experimental infection two types of birds were used. The experiment was carried out from 16th April to 30th May 2012. Fifteen pigeon birds (Columba livia domestica) five weeks old were used. The birds were divided into three equal groups. Group one was used as control, group two was inoculated with crude virus sample, group three was inoculated with the virus that was mixed with equal volume of anti-pigeon poxvirus hyperimmune serum (HIS) and incubated for one hour at 37 °C. Experimental infection was carried out by pulling out the feathers near peak area followed by application of (0.1) ml of the virus samples on such areas. The control birds were inoculated as the infected birds but with sterile PBS. Furthermore, infected and control groups were separated completely from each other and observed daily for development of clinical signs. Experimental infection in chicken: In the second experiment 45 chicken birds were used with no past history of infection with fowl or pigeon poxviruses. They were of 4-5 weeks old. Experimental birds were divided into three groups, 15 birds each and inoculated as shown in (Table, 1).

Table, 1: showed the experimental design of chicken inoculation with isolated pigeon pox virus.

Group's number	Number of birds		Method of inoculation	Dose of inoculum
	Control*	Infected		
Group 1	5	10	Feather follicle	0.1 ml
Group 2	5	10	Intravenous	0.1 ml
Group 3	5	10	Tracheal inoculation	0.1 ml

***The dose in control birds is the same as in infected but sterile normal saline was used instead of virus.**

Results and Discussion

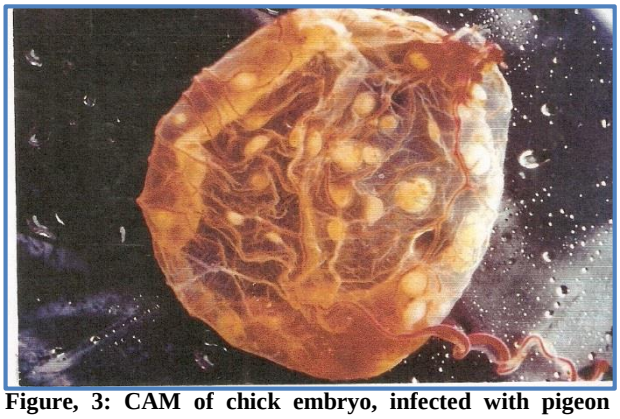
Clinical examination revealed that the head especially the area around peak was covered with papules and pustules. Some of these pustules were covered with thick yellow or brown scabs. Some other showed purulent secretion comes out of cracked scabs (Fig. 1). Also the infection appeared in vent area around the cloaca (Fig. 2).

Figure, 1: Pigeonpox, the head and especially the area around peak were infected with papules and pustules.

Figure, 2: Pigeonpox, the pock lesions in vent area around cloaca.

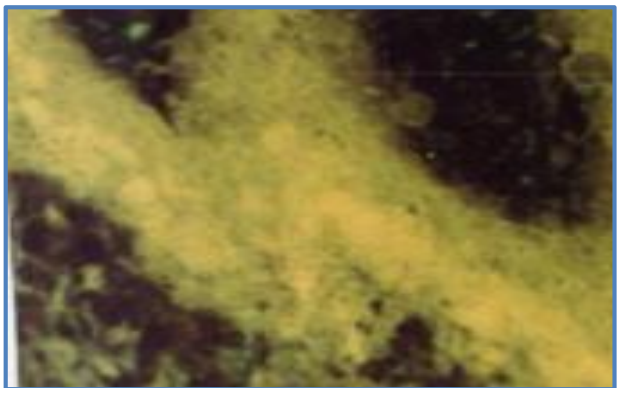
In the first passage in CAMs inoculated with processed samples, they showed single edematous and diffused pock lesions at the site of inoculation. On the second passage the pock lesions appeared little clearer than the first passage and many edematous pock lesions were observed. The third passage showed very clear, rounded opaque and separated typical pock lesions (Fig. 3). The fourth and fifth passages lesions were numerous and small in size. Inoculated chicken embryos started to die

from the third day post inoculation and all embryos died at the 6th days post inoculation during the first passage. In the second passage, the death started from the second day post inoculation and completely died five days post inoculation. All the 3rd, 4th, and 5th passages of inoculated chick embryos died at day four post inoculation.

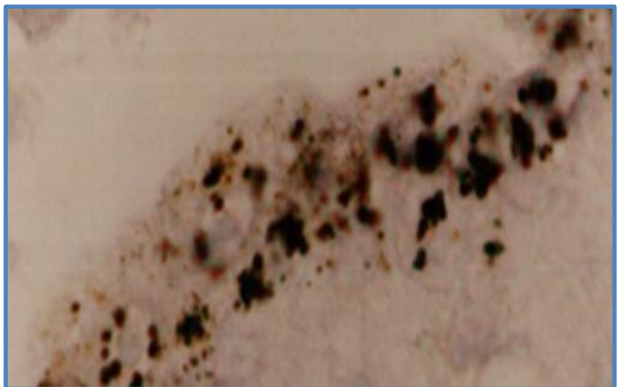


Figure, 3: CAM of chick embryo, infected with pigeon poxvirus. The pock lesions appeared as opaque rounded areas with different size.

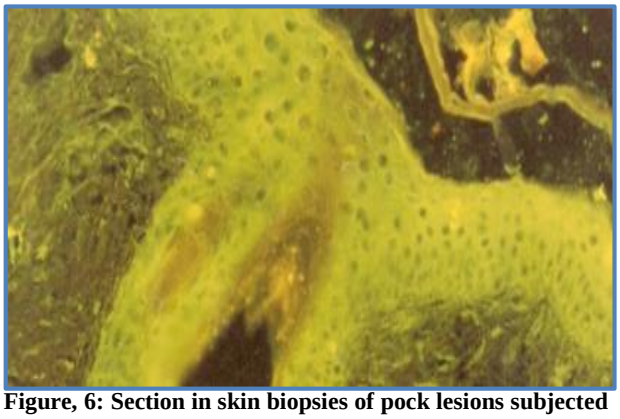
The results of viral titration of the virus from third, fourth and fifth passages on CAM of 11 days chick embryo, showed increase in one log in the titer starting from 1.5 X 10^{-4} Pock forming units/0.1 ml for the third passage into 2 X10^{-5} for the fourth passage and 1.25 x 10^{-7} for the fifth passage. The virus induced clear slide HA of RBCs collected from fowl, turkey and ducks. Infected CAM of chick embryo sectioned and stained in IIF showed yellow-green coloration of highly thickened and proliferated ectodermal layer (Fig. 4). Staining sections of infected CAM with IIP showed masses of deep and light brown coloration of thickened ectoderm (Fig. 5). Skin biopsies kept in 10% buffered formalin and processed for IIF test showed clear yellow green coloration of epidermis layer specially in the cytoplasm of infected cells while the nuclei appeared of dark coloration (Fig. 6). Subjecting of infected skin tissue biopsies to IIP showed dark brown coloration of cytoplasm of epidermis cells, while the nuclei appeared pale in color (Fig. 7). Histopatho-logical examination of infected skin biopsies stained with H and E stain showed localized proliferations of epithelial cells of the feather follicles or the skin. The affected cells became hyperplastic and hypertrophic as the increased rate of multiplication occurs in the basal germinativum layer of cells within the epithelium (Fig. 8).



Figure, 4: IIF test on infected CAM with pigeon poxvirus showed the ectodermal layer with yellowish-green coloration.

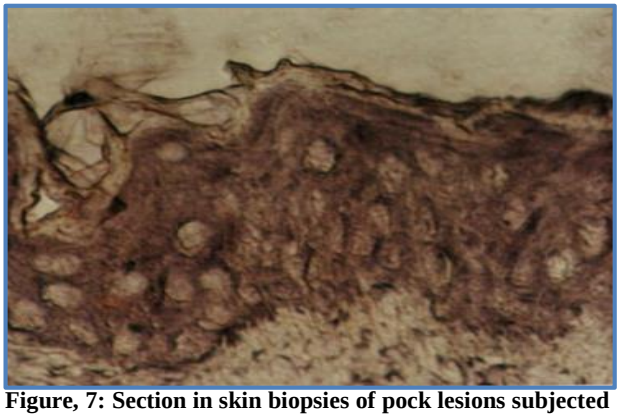


Figure, 5: IIP test on infected CAM with pigeon poxvirus showed dark brown coloration of infected ectodermal cells.

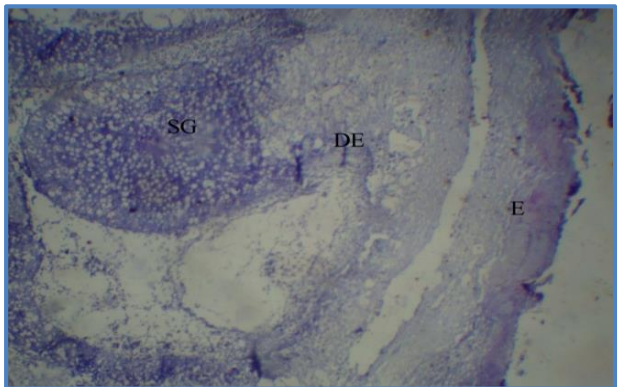


Figure, 6: Section in skin biopsies of pock lesions subjected IIF test showed the yellowish-green coloration of infected epidermis.

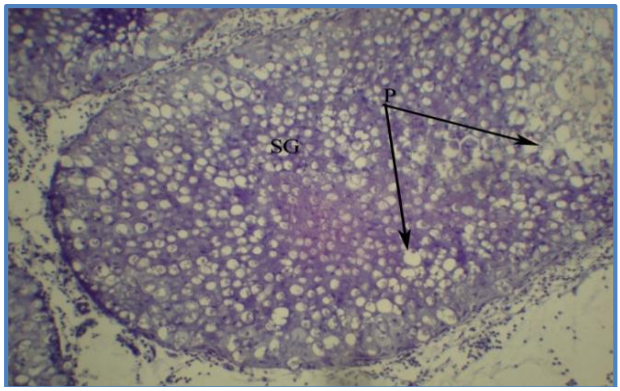
The section in warts showed enlarged epithelial cells of different lengths (Fig. 9 and 10), hyperplasia and necrosis (Fig. 11). At the border of necrotic foci, eosinophilic cytoplasmic inclusion bodies were observed (Fig. 12). With high magnification view of the same lesion, ballooning of epithelial cells was seen (Fig. 13).



Figure, 7: Section in skin biopsies of pock lesions subjected IIP test showed the dark brown color of the cells of infected epidermis. The nuclei appear pale while the cytoplasm is dark.

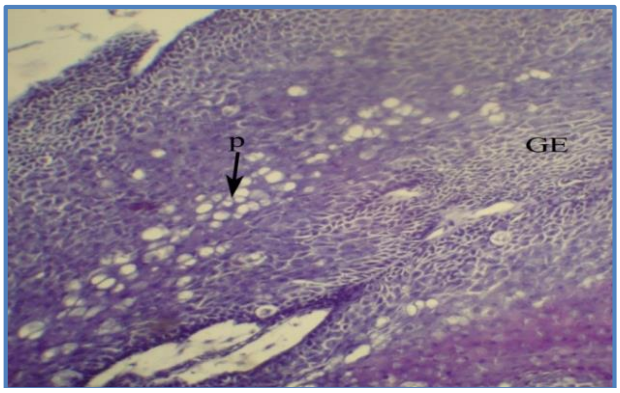


Figure, 8: Histological section of pigeon poxvirus infection of skin from the cloacal vent region of a naturally infected domestic pigeon (Columba livia domestica) illustrated in E. epidermis, DE, dermis and SG, stratum germinatum which that infected. H and E.

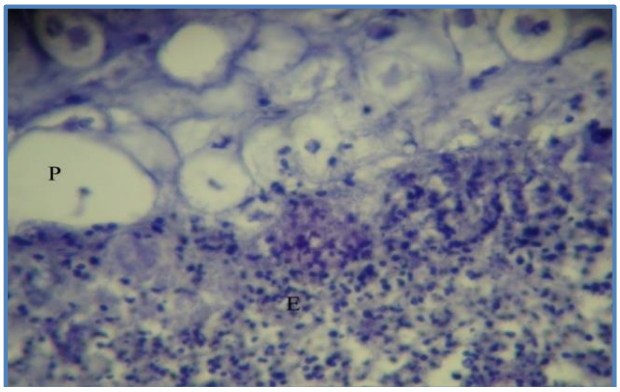


Figure, 9: Proliferation (P) of epithelial cell in SG, stratum germinatum. H and E.

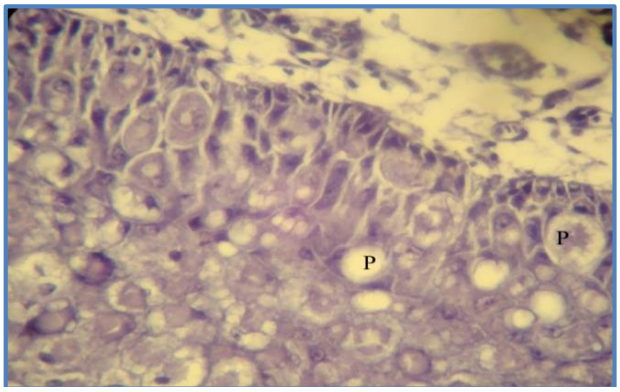
The results of experimental infection in pigeon showed that the virus induced moderate infection. Few vesicles and pustules were observed on the peak of three pigeon birds 9 days post infection while the other two birds showed such lesions 11 days PI (Fig. 14). Sixteen days PI, two birds died out of five while three of them survive with regression of pock lesion. Complete healing of such birds was completed 5 weeks post infection. One out of two dead birds showed severe pock lesions in the vent area 16 days PI (Fig. 15).



Figure, 10: Proliferation of the epithelial cell germinal epithelium (GE) of the skin due to the natural infection of pigeon with pigeon poxvirus. H and E stain.



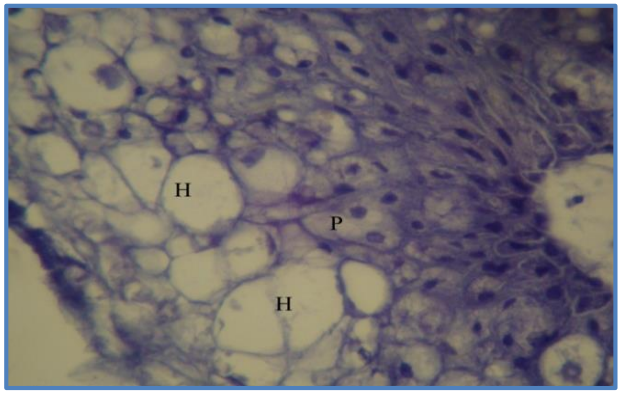
Figure, 11: Proliferation (P), hyperplasia and hypertrophy of stratified squamous epithelial cells of pigeon skin infected with pigeon poxvirus. High magnification view of the same lesion showing the ballooning of epithelial cells. H and E.



Figure, 12: Proliferation (P), hyperplasia and hypertrophy of stratified squamous epithelial cells of skin. H and E stain.

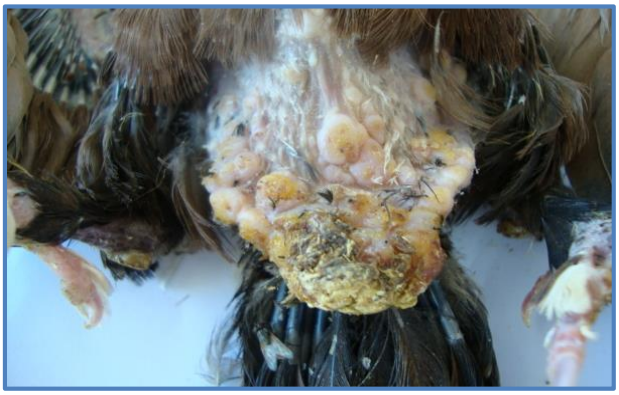
Pigeon group that infected with virus sample treated with HIS did not show any sign of pigeon pox infection, and all the five birds survived. In chicken, pigeon poxvirus generally showed mild infections. The virus induced mild and small pock lesions appeared 12 days PI of infected feather's follicles and only in five birds. Nearly similar finding were

observed in group 2, when very small pock lesions were observed on the face of infected birds 9 days PI. Group 3 of birds showed mild respiratory signs in three birds out of 10. All the live birds were completely healed 4 week PI.



Figure, 13: Proliferation (P), Hyperplasia (H), and hypertrophy of epithelial cells of skin. High magnification view of the same lesion showing the ballooning of epithelial cells. H and E Stain.

Figure, 14: Pock lesions appeared on the peak and eye lids 6 days PI.



Figure, 15: Severe pock lesions appeared on vent area 10 days PI.

Postmortem finding of the infected dead pigeons showed congestion of respiratory tract. The present case seems to be highly acute and most infected pigeons died. Sever cases of pigeon poxvirus were observed worldwide (16). The virus was successfully isolated in chorioallantoic membrane (CAM) of chick embryo. This method was successfully used for isolation of poxviruses (11, 17 and 18). CAM used to differentiate between several isolates of avipoxviruses from different avian species (19) the virus was identified by IIF and IIP in addition to experimental infection when the inoculum was mixed with HIS against pigeon poxvirus. Serological tests were generally used for diagnosis of bacterial and viral infections in addition to many other disease conditions (5 and 20).

In histopathological section, warts of pigeon pox infections were found to be similar to the findings of many authors, authors (21) explained that the hypertrophy and large granular acidophilic intracytoplasmic inclusions appear as the cells mature in layers of epithelium above the stratum germinatum through the infected epithelial cells to form "pocks". In addition (8) found in avian pox may have cutaneous lesions on not only exposed skin areas but also the feathered portions of the body. Also (17), found that the inclusion bodies become particularly evident in the epidermal cells during the subacute or chronic stages of the disease. However (22) observed rod- or brick shaped inclusions in the cytoplasm of hypertrophic epithelial cells that bore typical Bollinger bodies. The same authors also mentioned that Imperial Eagle (*Aquila heliaca*), and demonstrated more typical inclusion bodies. It is well known that avipoxviruses agglutinate RBCs of different bird species (9, 23 and 24).

In experimental infection, the virus appeared of moderate severity in pigeon while it did not induce clear pathogenic infection in chicken. These findings come in agreement with finding of (9 and 25). Experimentally infected chickens with pigeon pox virus by oral, intravenous or wing-web puncture routes did not show any pox lesions except for a "take reaction" at the inoculation site. The severity of infection in experimental birds was manifested with a descending magnitude after intravenous, oral and wing-web puncture inoculation. It could be concluded that the field isolates of pigeon pox manifested considerable host specificity to pigeons. The present isolate was of moderate severity to

pigeon and very mild to fowl. Furthermore, the death of two pigeons may be attributed to other factors; one of them was the possibility of contamination with pathogenic bacteria in pock lesion reported in vent area. In conclusion, the pigeon poxvirus was successfully isolated, identified and characterized from histopathological points of view. It is recommended to perform further studies on using such isolate for the purpose of vaccine preparation.

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عزل فيروس جدري الحمام من حالات إصابة شديدة في الحمام في محافظة ديالى، دراسة فيروسية ونسجية مرضية

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الخلاصة

عُزل فيروس الجدري من حمام مصاب إصابة شديدة بجدري الطيور من 64 طيراً في بعقوبة/ محافظة ديالى. نُمي الفيروس على الغشاء اللقانقي لأجنة الدجاج بعمر 11-12 يوم وحفز فيها تكوين افات جدري نموذجية. المقاطع المجهرية للأنسجة من النماذج المرضية والمصبوغة بصبغة الهيماتوكسلين-ايوسين أظهرت آفات مرضية لفيروس الجدري. كُشف عن الفيروس باستعمال مصل ممنع مضاد وباستعمال الإختبار غير المباشر للومضان الممنع وانزيم البيروكسديس. أظهر الفيروس قدرة على تلزين كريات الدم الحمراء لطيور الحمام والدجاج والتركي والبط. كما إنّ الإصابة التجريبية في الحمام أظهرت ان الفيروس متوسط الشدة المرضية في حين ظهر خفيف الشدة في الاصابة التجريبية في أفراخ الدجاج. نستنتج من الدراسة إمكانية عزل فيروس جدري الحمام بنجاح والتعرف على صفاته عن طريق المقاطع النسجية المرضية كما يمكن تحضير لقاح ضد المرض.

الكلمات المفتاحية: جدري الحمام، جدري الطيور، اختبار الومضان الممنع، اختبار البيروكسديس الممنع.