Dept. of Poultry Diseases,
Fac. of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. S. Mousa.

IDENTIFICATION AND CHARACTERIZATION OF POXVIRUS ISOLATED FROM TURKEYS

(With 4 Tables and 5 Figures)

By

K.S. EL-ZANATY

(Received at 24/2/1990)

وصف وتصنيف فيروس الجدرى المعزول من الرومـــــــــى

كمال الزناتـــــــــــــى

خلال صيف عام ١٩٨٨م ظهر الإصابة بوباء فيروس الجدرى في الرومى في محافظة سوهـــاج بصورتيه الجلدية والعينية وقد تم عزل ثلاث عترات من أماكن متفرقة · تم تنمية ومعايرة هـــذه العترات على أغشية أجنة الدجاج · وقد أظهرت بثرات مميزة على الأغشية · أجريت التجـارب المعملية للتعرف على فيروس الجدرى وكذلك الفحوص التجريبية على كتاكيت عمر يوم واحـــد الرومى ، الدجاج ، البط ، الحمام · وقد تم توصيف الأعراض الظاهرية والهستوباثولوجية ·

SUMMARY

During summer 1988, natural outbreaks of poxvirus infection in turkeys with severe cutaneous and ocular lesions in Sohag Province was described. Three poxvirus-isolates were recovered. All virus-isolates were propagated and titrated on chorioallantoic membrane (CAM) of 11-day-old embryonated chicken eggs (ECE). The biological and some of the physico-chemical properties of the virus isolates were studied. The host spectrum of the isolated virus was studied through experimental infection of one-day-old chicks, chickens, turkeys, ducks and pigeons. The gross and microscopic feature in experimental infection were described.

INTRODUCTION

Fowl and turkey poxvirus infections are widely spread in Egypt (EL-SABBAGH, 1962; ISHAK, 1977). These diseases are considered of economic importance owing to the relatively high mortality and drastic drop in egg production they produce in susceptible birds. Fowlpox virus is considered the most extensively studied member of Avipox viruses (ANDREWS and PEREIRA, 1972), while turkey pox virus seems less extensively studied (BUXTON and FRASER, 1977). The present study describe natural outbreaks of pox virus infection among turkeys and reports biological and some of physico-chemical properties of isolates in addition to virological and pathological characteristics induced by experimental infection in some avian species.

MATERIAL and METHODS

History of the outbreaks:

Turkey flocks in six villages Located in different localities in Sohag Province showed lesions of poxvirus infection. Average number of turkeys 750-1100 birds/Village and the age of the birds ranged from 4 to 18 months. The morbidity rate among turkeys was 44-56%. Two typical forms (Cutaneous and Ocular) of pox virus infection were observed (Figs. 1-3). The ocular lesions were more evident and severe.

Specimens :

Scabs and tissues from cutaneous and ocular lesions were collected during natural outbreaks of pox infection in turkeys. Material was ground and a 20% suspension was made in sterile normal saline containing penicillin and streptomycin. The suspension was then centrifuged for 20 minutes at 2.000 rpm and the supernatant fluid was used as inoculum.

Chicken embryos and one-day-old chicks :

These were obtained from the farm of Faculty of Agriculture, Assiut University.

Virus isoaltion :

11-day-old chick embryos were inoculated on the chorioallantoic membrane (CAM) with 0.1 ml of the inoculum by the coventional drop membrane method. Six days after inoculation the CAMs were collected and examined for characteristic pock lesions. The infected CAMs were divided into two portions, one used for further passages and ager gel preciptation test and the other processed for histopathological study.

Virus titration :

Titration of virus isolates was done by CAM inoculation with 0.1 ml of virus dilution. Five eggs were used for each dilution. The highest dilution of the virus giving five or more pocks on CAM 120 hours postinoculation was the titre of the virus and expressed as pock forming units (PFU) per 0.1 ml (KAR and PATHAK, 1980).

Haemagglutination (HA) tests :

The virus-isolates were tested for HA activity, after ANNON (1971).

Agar gel precipitation (AGP) test :

The test was conducted as described by CUNNINGHAM (1966). Antigen was used in the form of previously fowl pox virus infected CAM suspension as control. Hyperimmune sera against fowl pox virus prepared in rabbit according to GISPEN (1955) was used as control.

Heat stability :

The thermostability of the virus-isolates was done after HASS and DARDIRI (1968). They were exposed to 56 C for 15, 30 and 60 min. Additional samples were left at room temperature as control. Treated and untreated control samples were checked for infectivity by titration onto CAM of ECE.

Ether and chloroform sensitivity :

The effect of 10% diethyl ether and 20% chloroform on virus-isolates was studied after ANDREWS & HORSTMANN (1949) and FELDMAN & WANG (1961). Treated and untreated control samples were checked for virus-infectivity as above.

Turkeys, chickens, ducks and pigeons :

These were obtained from the local market, about 4 months age. They were free from any pox lesions. Individual serumsamples were checked for precipitating antibodies by AGP test before being inoculated.

Pathogenicity tests :

One isolate (No. 11) was used as inoculum at the 2nd egg passage level in the form of CAM suspension containing 1.3×10^3 PFU/0.1 ml for inoculation of one-day-old chicks, chickens, turkeys ducks and pigeons. Birds of the same age and from the same source were used as a control group. Number of inoculated birds, route of inoculation, dose of inoculum/bird are shown in Table (1).

Histopathology :

Infected CAMs showing pock lesions and also wing web lesions from inoculated chickens were processed as usual for histopathology in Department of pathology, Fac. Vet. Med. Assiut University.

Table (1): Experimental infection of different species with poxvirus isolate (No. II).

Species	Route of inoculation	No. of bird inoculated	Dose ml/bird
One day-old chicks	- I/V (brochial wing vein)	10	0.05
	- Swabbing defeathered skin over pectoral muscle and wing web stabbing.	10	0.1
Chickens	- I/V (Wing vein)	3	0.2
	- Scarification comb & Wattles and Wing web stabbing	3	0.5
Turkeys	- I/V (Wing vein)	2	0.2
	- Scarification snood & dewlap and inside the thigh.	2	0.5
Ducks -	- I/V (Saphenous vein)	2	0.2
	- Swabbing defeathered skin on thigh and wing web stabbing.	2	0.5
Pigeons	- I/V (wing vein)	3	0.1
	- Wing web & feather follicle methods	3	0.2

I/V = Intravenous.

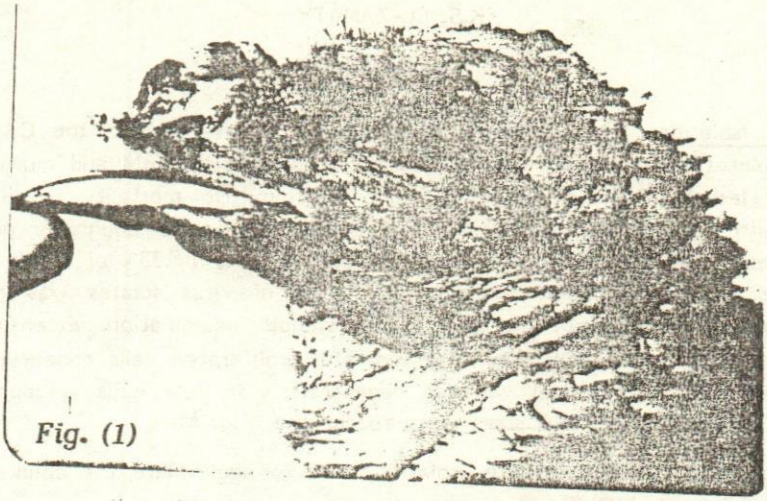

Fig. (1)

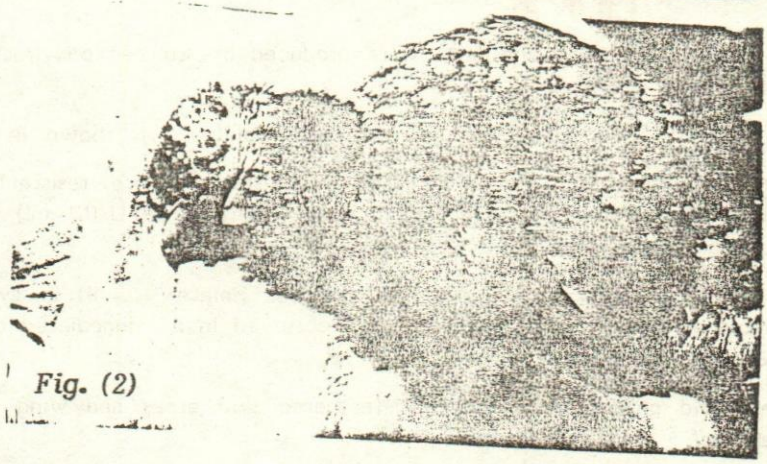

Fig. (2)

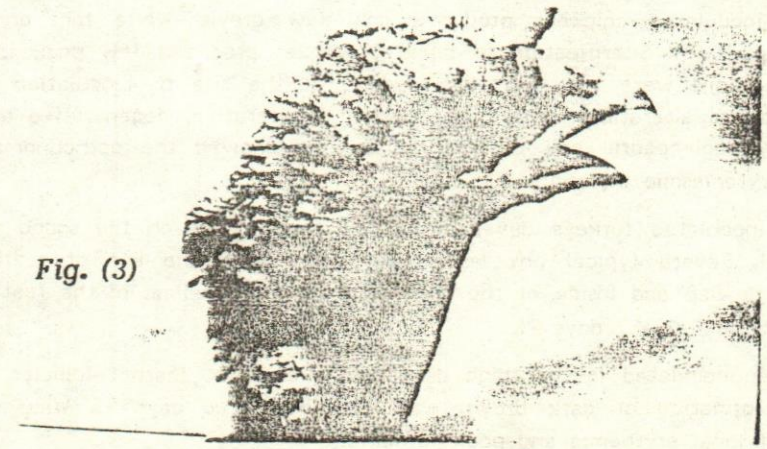

Fig. (3)

Fig. (1-3): Naturally infected turkeys with poxvirus. Note: Severe cutaneous and ocular lesions, dark brown wart like nodules covering head region, unilateral or bilateral blindness.

RESULTS

Virus isolation: Three poxvirus isolates were recovered into the CAM of ECE. All virus isolates produced on the 1st passage thickening of CAM and multiple greyish white pock lesions. Pock size ranged from 2-3 mm. No mortality or lesions were observed with any of the embryos. Clear characteristic and countable pock lesions were seen on CAM after 6th day of inoculation. However in 33% of CAMs coalesence of such lesions was constant findings. The titre of virus-isolates was reduced by chicken embryos passages (Table 2). On microscopic examination, extensive proliferation of the ectodermal cells was observed. The proliferated cells appeared as multiple projections and manifested vascular degeneration. In few cells acidophilic, intracytoplasmic inclusion of various sizes were recognized (Fig. 4).

HA activity: The three virus isolates failed to agglutinate the chicken erythrocytes.

AGP test: Clear precipitine lines were produced by isolated poxviruses infected CAMs against control antisera.

Heat stability: The effect of heat on virus-isolates was shown in Table (3).

Ether and chloroform resistance: The virus-isolates were resistant to ether and chloroform treatment as no reduction in virus infectivity (PFU/0.1 ml) was detected in comparison to untreated control samples.

The results of pathogenicity tests of poxvirus isolate (No. II) in avain species are summarized in (Table 4). No lesions were observed in I/V inoculated one-day-old chicks, ducks, and pigeons.

One-day-old chicks inoculated in defeathered skin areas and wing web developed whitish foci 5 days postinfection (PI).

I/V inoculated chickens produced only few greyish-white foci on comb and wattles 7 days. PI. Scarification of comb & wattles produced few pock lesions while wing web lesions were tiny wart-like nodules at the site of inoculation 9 days PI. Histopathological alterations were manifested in proliferative, degenerative and necrotic changes, the polyheadral cell of stratum spirosum showed the pathognomonic acidophilic intracytoplasmic inclusions (Fig. 5).

IV/ inoculated turkeys developed a few small pocks on the snood and dewlap 12 days PI. Severe typical pox lesions developed along the line of scarification on the snood, dewlap and inside of the thigh with marked swelling of the feather follicles 4-5 times normal size 7 days PI.

Ducks inoculated in the thigh developed swelling of feather follicles 2-3 normal size with formation of dark brown wart like nodules 8 days PI. Wing web lesions consisted of local erythemia and pock formation.

Pigeons inoculated by wing web and feather follicle method produced typical cutaneous lesions 10 days PI at the site of inoculation.

Table (2): Titration of virus isolates by pock count method after 120 hours postinoculation.

Virus isolate number	2nd virus passage					3rd virus passage				
	Average pock count per virus dilution				Titre of virus 0.1 ml. (PFU / 0.1 ml.)	Average pock count per virus dilution				Titre of virus 0.1 ml. (PFU / 0.1 ml.)
	10^{-1}	10^{-2}	10^{-3}	10^{-4}		10^{-1}	10^{-2}	10^{-3}	10^{-4}	
I	18	2	-	-	1.8×10^2	5	-	-	-	0.5×10^2
II	39	3	1	-	1.3×10^3	12	2	-	-	1.2×10^2
III	11	1	-	-	1.1×10^2	-	-	-	-	-

Table (3): The effect of heat on virus-isolates.

Virus-isolate number	Heat treated virus after 15 min.	30 min.	60 min.	Untreated control virus
I	*1.6×10^2	0.2×10^2	0	1.8×10^2 PFU
II	1.2×10^3	0.7×10^2	0	1.3×10^3 PFU
III	0.9×10^2	0.3×10^2	0	1.1×10^2 PFU

* Titre of the virus-isolate/0.1 ml (PFU/0.1 ml).

Table ( ): Pathogenicity of pox virus-isolate No. II in avian species.

Route of inoculation	Avian species				
	Turkeys	chickens	One-day-old chicks	Pigeons	Ducks
C	+++	++	++	+++	++
I/V	+G	+G	-	-	-

C = Cutaneous. I/V = Intravenous.
+++ = Severe local pox lesions ++ = Moderate local pox lesions
+G = Generalized infection. - = No lesions.

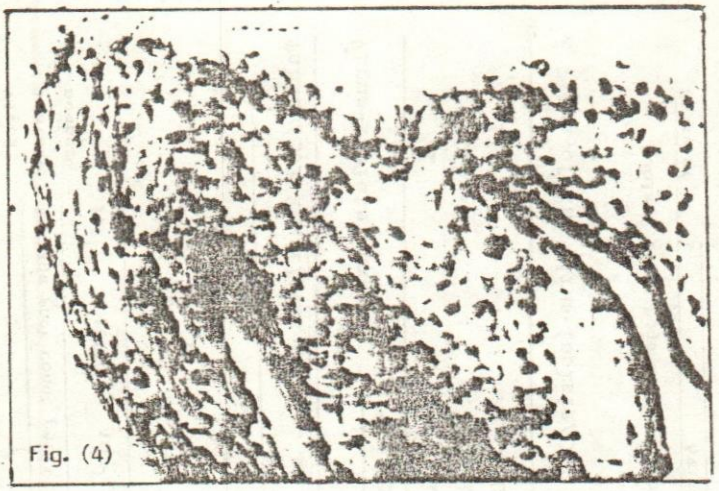

Fig. (4): CAM section showing excessive hyperplasia and intracytoplasmic inclusions produced by isolate poxvirus (No. II) after 120 hours of infection.

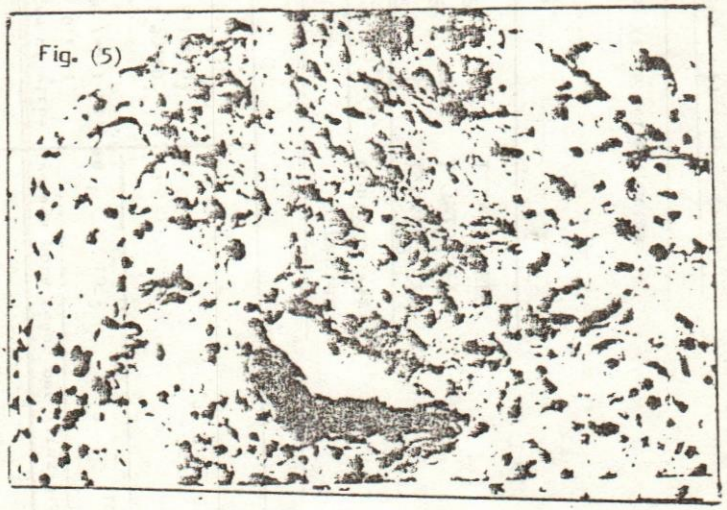

Fig. (5): Section in wing web infected with poxvirus isolate No. II. Note Intracytoplasmic inclusions. (H & E).

DISCUSSION

In the present investigation, the clinical lesions of the naturally infected turkeys as well as the biological and physicochemical properties, virological studies, pathogenicity tests and histopathological findings revealed the prevalent widespread natural poxvirus infection in turkeys which represent the first record in Upper Egypt. TANTAWY et al., 1978, isolated and characterized poxvirus from turkeys during an outbreak in Giza district. Oral membrane lesions could not be seen in the naturally infected turkeys in these outbreaks.

Blind passages of virus-isolate (II) in ECE resulted in reduction of virus titre (Table 2) which indicate the less susceptibility of ECE to poxvirus isolated from turkeys. CUNNINGHAM, 1978 reported that avian pox isolates frequently infect a heterologous host but are usually most pathogenic in the species from which they were isolated. Pathogenicity of the isolated poxvirus (isolate II) in one-day-old chicks, chickens, turkeys, ducks and pigeons produced cutaneous lesions with little variations (Table 4). These observations are in close agreement with those reported by TRIPATHY and CUNNINGHAM, 1984. I/V inoculted turkeys developed no diphtheritic lesions which disagreed with DAVIES and MUNGAL, 1978. No lesions were observed in I/V inoculated chicks, while TANTAWY et al., 1978 reported that both cutaneous and dipheretic lesions developed in chicks. I/V inoculated with turkey poxvirus. I/V inoculated chickens showed no mortality which differ from those reported by KHEIR EL-DIN et al., 1978.

The histopathological findings are in general agreement with PANDY and MALLICK, 1975; KAR and PATHAK, 1980 and ELAMIN et al., 1980.

On the basis of low susceptibility of ECE to poxvirus-isolate (No. II), no mortality or lesions in any of inoculated embryos (KAR and PATHAK, 1980), severe cutaneous lesions in inoculated turkeys (CUNNINGHAM, 1978) and susceptility of ducks to experimental infection (MAYR, 1963; GELENCZEI & LASHER, 1968 and TRIPATHY & CUNNINGHAM, 1984) it is concluded that the isolated virus (No. II) was turkey poxvirus.

ACKNOWLEDGMENT

I wish to thank Prof. Dr. Bayoumi, Professor of Vet. Pathology, Fac. Vet. Med. Assiut University.

REFERENCES

Andrews, C.H. and Horstmann, D.M. (1949): The susceptibility of viruses to ethyl ether. J. Gen. Microbiol. 3: 290-292.

Andrews, C.H. and Pereira, H.G. (1972): Viruses of Vertebrates. 2nd Ed. Williams and Wilkins Co. Baltimore.

Annon (1971): Methods for examining poultry biologies and identifying and quantifying of avian pathogen. National Academy of Science, Washington, D.C.

Buxton, A. and Fraser, G. (1977): Animal Microbiology. Blackwell Scientific Publication.

Cunningham, C.H. (1966): A laboratory Guide in virology 6th ed. Burgess Publication Company, Minneapolis.

Cunningham, C.H. (1978): Avian pox in: Diseases of poultry, 7th Ed. M.S. Hofstad, B.W. Calnek, C.F. Helmboldt, W.M. Reid, and H.W. Yoder. Jr., eds. Iowa State University Press, Ames. Iowa pp. 597-609.

Davies, F.G. and Mungal, J.M. (1978): Turkey pox. Vet. Rec. 103. P. 104.

Elamin, G.; Tageldin, M.H. and Babiker, S.H. (1980): Fowl pox virus in the Sudan. Avian Dis. 24: No. 3, 763-770.

El-Sabbagh, A.H. (1962): Some studies on an Egyptian strain of fowl pox virus. M.D. Vet. Thesis, Cairo University.

Feldman, H.A. and Wang, S.S. (1961): Sensitivity of various viruses to chloroform. Proc. Soc. Exptl. Biol. and Med. 106, 736-740.

Gelenczei, E.F. and Lasher, H.N. (1968): Comparative studies of cell-culture propagated avian poxviruses in chickens and turkeys. Avian Dis. 12: 142-150.

Gispen, R. (1955): Analysis of poxviruses antigens by means of double diffusion. A method for direct serological differentiation of cowpox. J. Immunol. 74: 134-141.

Hass, W.R. and Dardiri, A.H. (1968): Some properties of the virus of duck plaque. Arch. Für die Gesamt. Virus Forschung 24: 148-153.

Ishak, A.M. (1977): Studies on avian pox vaccines. M.V.Sc. Thesis, Cairo University.

kar, B.C. and Pathak, R.C. (1980): Cultivation and titration of some avian pox viruses in developing chick embryo. Indian Vet. J. 57, 99-101.

Kheir Eldin, A.M.W.; Tantawi H. and Youssef Y.I. (1978): Clinical and immune response of chicken to two field isolates of pox virus recovered from chickens and turkeys. J. Egypt. Vet. Med. Assoc. 38: No. 3, 27-33.

Mayr, A. (1963): Neue Verfahren Für die Differenzierung der geflugel pocken-viren. Berlin. Muench Tierarztl. Wochschr. 76: 316-324.

Pandey, K.D. and Mallick, B.B. (1975): Cultivation of avian poxes in developing chick embryo. Indian J. Anim. Hlth. 14: 99-101.

Tantawy, H.; Kheir El-Din A.M.W. and Youssef, Y.I. (1978): Studies on two isolates of pox virus recorded from chickens and labortory characterization. J. Egypt. Vet. Med. Assoc. 38, No. 3, 17-25.

Tripathy, D.N. and Cunningham, C.H. (1984): Avian pox. In Diseases of poultry, 8th Ed. M.S. Hofstad, B.W. Calnek, C.F. Helmboldt, W.M. Reid, and H.W. Yo der, Jr., eds. Iowa State University Press, Ames, Iowa. pp. 524-534.