Department of Virology,
Animal Health Research Institute, Dokki, Cairo.

SOME STUDIES ON GOAT POX VIRUS

(With 2 Photo)

By

AMAL M.A. RAOF; NAHED A. MAHMOUD and A.S. AHMED*

* ELISA unit, Animal Health Research Institute Dokki, Cairo.

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بعض الدراسات على فيروس جدري الماعز

أمل مختار عبد الرؤف ، ناهد احمد محمود ، أحمد سعيد احمد

هـذا البحـث يهدف إلي دراسة الصفات الفيزيائية – الكيميائية والبيولوجية لعترة محلية من جـدري الماعـز معزولة من محطة سخا- كفر الشيخ ٢٠٠١ وكذلك لدراسة اختبار التعادل العكسي بين الفيروس وفيروس جدري الأغنام. تم تمرير الفيروس وقياس عياريتة على خلايا VERO ونتج عن ذلك .CPE مميزة لفيروس الجدري. لدراسة تأثير الحرارة على عيارية الفـيروس وجد انه ثابت عند درجتي حرارة الغرفة ، ٣٧ درجة مئوية لمدة ٧ أيام متصلة، بيـنما الحرارة العالية ٥٦ درجة مئوية لمدة ساعتين تنقص من عيارية الفيروس. عند تأثير الأشعة فوق البنفسجية على الفيروس وجد انه حساس لهذه الأشعة. بالنسبة لدراسة المؤثرات الكيميائية وجد تأثير ٠,٥% فورما لين ينقص من عيارية الفيروس تدريجيا ليصل ١٠^{٥} بدلا مـن ١٠^{٥}بعد ٢٤ ساعة . بالنسبة لتأثير الأثير وجد انه ينقص من عيارية الفيروس تدريجيا ليصل إلى ١٠^{٢} بعد ٢٤ ساعة. أما بالنسبة لبرمنجنات البوتاسيوم والكحول على العيارية عند التعرض لهما لمدة ساعة. اختبار التعادل العكسي أوضح أن هناك علاقة بين فيروس جدري الماعز وجدري الأغنام. عند القيام باختبار التلزن الدموي وجد أن الفيروس يقوم بعمل تلذن لكرات الدم الحمراء للدواجن.

SUMMARY

This work aimed to study the effect of physico-chemical agents on the stability and biological properties of local strain of goat pox virus (GPV) as well as perform haemagglutination test and cross neutralization test between this virus and sheep pox virus. The virus was propagated on VERO cells and produce CPE specific to pox virus. On study the effect of heat on the virus, the results showed that GPV was stable at both room temperature and 37°C for 7 successive days while the virus reduced its infectivity after 2 hours at 56°C; the virus was stable at 4 repeating cycles of freezing and thawing. On studying the effect of ultra

violet rays UVR the result showed that the virus was sensitive to UVR. Regarding to the effect of chemical agents the results revealed that; the effect of 0.5% formalin on the virus infectivity resulted in decreased virus infectivity gradually from 10^5 to $10^{0.5}$ $TCID_{50}$ /ml over 24 hours of treatment; the effect of 20% ethyl ether on the virus infectivity the result showed that virus infectivity decreased gradually till reach 10^2 $TCID_{50}$ /ml. over 24 hours of treatment; the effect of both 50% alcohol and 0.01% potassium permanganate on the infectivity, revealed that virus was inactivated within one hour of treatment at room temperature. Regarding to cross neutralization test; there was a cross neutralization between goat pox and sheep pox virus which concluded that there was antigenic relationship between the two viruses. The result of Haemagglutination test revealed that the isolated virus agglutinate RBCs of chickens.

Key words: *Virology, pox, goat.*

INTRODUCTION

Goat pox virus (GPV) is a member of Capri poxvirus genus of the *Poxvirdae* Family, which is etiologic agent of important disease of goats in northern and central Africa, south west and central Asia, and Indian sub- continent Capstick (1961) and Rao and Bandyopadhyay (2000).

Pox virus affect most animal species causing considerable economic losses and high mortality in young animals Mathew (1982).

The members of family *Poxvirdae* shared in the morphology, presence of double stranded DNA in the viral genome, antigenic relationship to other pox viruses, sensitivity to lipid solvents, acid liability, and induction of skin lesions Cottral (1978).

Goat pox virus isolated during outbreak of pox infection among goats, the isolated virus grew on the chorio-allantoic membrane of developing chick embryos producing generalized large pocks and in primary lamb testis cell cultures Tantawi *et al.,* (1980).

Outbreaks of goat pox occurred among 60 adult and young goats with wide distributions of pox lesions on the bodies of the animals, the sever involvement of mucus membranes of the muzzle, eyes and nostrils give rise to acute respiratory distress and systemic reaction Mohamed *et al.,* (1982).

Capri pox virus was transmitted by contact to sheep and goats kept with infected animals. The transmission of Capri pox to sheep was

also using an aerosol suspension. The incubation period for Capri virus infection in sheep and goats was approximately 8 to 12 days Kitching and Taylor (1985).

Goat pox can survive in scabs materials for period of 3-6 months Singh *et al.*, (1979), Thus recovered animals become a source of infection to susceptible population which they come in contact during seasonal grazing and trade movements Davies (1981).

Studies of various physico chemical properties of ovine pox virus showed that virus was susceptible to the action of heat at 50°C for 30 and 60 minutes, pH 3, ether and chloroform Das and Mallick (1985).

The suspension of pox viruses agglutinated erythrocytes of turkeys and fowls Anthony *et al.*, (1970). The Haemagglutination inhibition titers in sera from experimentally infected animals with pox virus were highest after 28-35 days post infection Rao and Adhakha (1970).

The inclusion bodies of sheep pox viruses, were of DNA and the viruses similar to that of lumpy skin disease in many characters. It was possible to cross protect both species with virus of sheep or goat origin Davies (1976).

There were antigenic relationships among sheep pox, goat pox and CPD viruses serologically using soluble antigens partially purified by DEAE cellulose chromatography Rao *et al.*, (1984).

The present work aimed to study the physico –chemical and biological properties of local isolate of goat pox isolated from Sakha station and Kafr El Sheekh provinces, as well as study the cross neutralization between this isolate and sheep pox virus.

MATERIALS and METHODS

1-Virus:

Local isolate of goat pox (GPV) isolated from Sakha station and Kafr EL Shekh provinces (Nawal *et al* 2001) which was isolated on 11 days old ECE via CAM route Joshi *et al.*, (1996). The virus was propagated in Vero cells for three passages. The virus was titrated according to Reed and Meunch (1938) and it was 10^5 $TCID_{50}$/ml.

2- Cell cultures:

VERO cells (Green Monkey Kidney cells), at AHRI as mono cell cultures were grown in Eagle's MEM with 10% new born calf serum. These cells were used for propagation and titration of GPV according to Katiyer and Soman (1986).

3-Rabbit hyper immune serum against Goat pox (RHIS-GPV):

Prepared according to (Davies, 1982) at Animal Health Research Institute AHRI, Virology Department, Dokki.

4- Sheep pox virus and hyper immune sera against sheep pox:

were obtained from the Pox Department, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo. Which were used in cross neutralization test.

5-Cross neutralization test:

It was performed between local isolate of goat pox virus and Sheep pox virus using their hyper immune sera prepared in rabbits, in which the virus –serum mixture were kept for 2 hours at 37°C according to Boulter (1957).

6- Haemagglutination test:

The test was performed on goat pox virus using erythrocytes of chickens according to Anthony *et al.*, (1970).

7- Chemical Reagents:

A) Formalin:

It was obtained as formaldehyde solution, which mixed with virus to give final concentration of 0.5%.

B) Diethyl ether:

it was prepared at a final concentration of 20% in virus.

C) Alcohol:

It was prepared at a concentration of 50% in virus as inactivating agent.

D) Potassium Permanganate:

$Kmno_4$ was prepared as 0.01% concentration as inactivating agent.

8- Ultra Violet Rays (UV):

It was used according to Kleezkowski (1959) where certain UV lamp with common wave was used in tissue culture cabinet on the distance of 30 cm on virus at petri dish with different times.

9- Effect of heat:

Effect of heat on the virus was performed at various degrees: room temperature, 37, 56°C, it was achieved according to William (1959).

RESULTS

Virus propagation and titration:

The local strain of goat pox virus was isolated from Sakha and Kafr EL Sheekh Province among fenlindi goats showed symptoms of goat pox (Nawal *et al.*, 2001), the virus was isolated on chorio-allantoic membranes (CAM).

The isolated virus was propagated on VERO cell cultures, the virus produced a cytopathic effect (CPE) that characterized by cell rounding and detachment.

These results represented in photos 1, 2 (photo 1 represented normal VERO cells and photo2 represented VERO cells showed CPE).

The virus was titrated on the same cells and virus titer was 10^5 $TCID_{50}$/ml.

Photo 1: VERO cells showed normal sheet.

Photo 2: VRRO cells showed CPE of goat pox.

Cross neutralization test:

Rabbits were used for preparation of hyper immune serum against local isolate of goat pox virus. The results showed that there was cross neutralization between goat pox and sheep pox viruses (each virus type had reacted with homologous and heterolgous rabbit hyper immune sera).

Haemagglutination (HA) test:

The isolated virus agglutinated RBCs of chickens at room temperature but with a low titer (1/8) and did not agglutinate RBCs of sheep.

Physico- chemical characters:

The study of the physico chemical properties of local isolate of goat pox virus, showed that:

The effect of heat on the infectivity of GPV:

1- The effect of heat at room temperature (20°C) for 7 successive days showed that the local isolate of GPV was stable.
2- The effect of heat at37°C for 7 successive days showed that the local isolate of GPV was stable.
3- The effect of heat at 56°C for 30 minutes, 1hour and 2 hours showed that the local isolate of GPV was relatively heat stable to this high temperature for up to 1hour and showed slow reduction in its infectivity from 2nd hour of exposure.
4- The effect of repeated cycles of freezing at -20°C and thawing at room temperature for 4 times showed that the local isolate of GPV was stable.

The effect of chemical reagents on the infectivity of GPV:

1- The effect of 20% ethyl ether on the infectivity of local isolate of GPV for intervals 6, 12, 18, 24 and 30 hours, showed that the infectivity decreased gradually by increasing the time of exposure till reaching 10^2 $TCID_{50}$ /ml over 24 hours.
2- The effect of 0.5% formalin on the infectivity of local isolate of GPV for time intervals of 6,12,18,24 and 30 hours, showed that the infectivity decreased gradually by increasing time of exposure until reaching $10^{0.5}$ $TCID_{50}$/ml over 24 hours.
3- The effect of 50% alcohol on the infectivity of local isolate of GPV for intervals 30 minutes, 1 hour and 2 hours, showed that the virus was inactivated within 1 hour at room temperature.
4- The effect of 0.01% potassium permanganate on the infectivity of local isolate of GPV for time intervals of 30 minutes, 1 hour

and 2 hours, showed that virus was inactivated within 1 hour at room temperature.

The effect of Ultra Violet rays on the infectivity of GPV:

The effect of UV rays at time intervals of 30 minutes, 1 hour and 2 hours, showed that the virus infectivity decreased gradually by increasing time of exposure which revealed that virus was sensitive to UVR.

DISCUSSION

Goat pox, is a notifiable disease in most countries of the world, many researches isolated Capri pox virus from sheep and goats in Egypt Soad *et al.*, 1996, as well as Capri pox antibodies also detected in sera of sheep and goats Rizkaallah 1994. In Egypt, pox is one of the most serious contagious diseases causing high mortality and great economic losses among susceptible animals as referred by Sabban 1960.

In this work we studied the effect of physical and chemical agents on the stability of local isolate of goat pox virus that showed several signs of respiratory manifestations and skin nodules at Sakha and Kafr EL Sheikh provinces (Nawal *et al.*, 2001). As well as studying the biological characters of the virus on the cell lines, and preparation of hyper immune serum in rabbits which used for cross neutralization test.

The local isolate of goat pox virus was propagated on VERO cells which considered the most suitable cells for pox virus Katiyer and Soman (1986), inducing CPE within 6 days which characterized by rounding and detachment (photo 1,2) and this result was agreement with Singh and Rai (1991) and Maity *et al.*, (1997).

The effect of temperature at different degrees on the stability of virus, showed that the virus was stable at both room temperature and at 37°C for 7 successive days while at 56°C for 30 minutes, 1 hour and 2 hours showed that the virus was reduced in its infectivity at 2^{nd} hour of exposure, this results confirmed the sensitivity of GPV to the heat while the virus was stable at 4 cycles of freezing and thawing. These results were agreement with Das and Mallick (1985).

The effect of 20% ethyl ether on stability of virus at intervals 6,12,18,24,30 hours results showed that the virus titer decreased gradually by increasing the time of treatment until reached to 10^2 $TCID_{50}$/ml, These results confirmed that GPV was sensitive to ether and these results were agreement with Plowright and Ferris (1959).

The effect of 0.5% formalin on the infectivity of virus at time intervals of 6,12,18,24,30 hours, showed that the virus infectivity

decreased gradually by time of treatment until virus reaching $10^{0.5}$ $TCID_{50}$/ml over 24 hours of treatment.

The effect of 50% alcohol, 0.01% potassium permanganate at time intervals of 30 minutes, 1 hour and 2 hours on the infectivity of virus, showed that virus was inactivated within one hour at room temperature, these results confirmed that virus was sensitive to alcohol and Pot. Permanganate and these results were agreement with Pandey and Sengh (1970 a).

Results of the effect of ultra violet rays on the infectivity of GPV showed that, virus was sensitive to UVR and this denoted that virus is easily affected by direct sun light.

The results referred to the antigenic relationship, there was cross neutralization reaction seen in neutralization test, and this result was agreed with Kitching and Tylor (1985).

The results refereed to the haemagglutination property of the goat pox virus showed that virus haemmaglutinate RBCs of chickens at room temperature while can not haemagglutinate sheep RBCs and these results were agreed with Nakano (1979).

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