

ISOLATION, CULTIVATION AND CHARACTERIZATION OF CAMEL POX VIRUS (*)

By

H. Ramyar and M. Hessami

Introduction

Camel pox infection has been previously described by a number of workers (1, 2, 3, 4, 5, 6 and 7), but the available literature shows that no attempt has been made to study the different properties of the causative agent.

This paper reports results of work carried out during the past two years on three different isolates of camel pox virus (CPV) harvested from naturally infected camels.

Material and Methods

Tissue culture

Monolayer tissue cultures of lamb kidney (LK) cells were prepared as previously described (8). The cells were grown in Hanks' balanced salt solution containing 10% unheated calf serum and 0.5% lactalbumin hydrolysate and then maintained in VM₃ (10).

Virus

Three isolates of tissue culture adapted CPV which had been carried through 10 subcultures were used. They were obtained from vesicles and crusts which were harvested from natural outbreaks of pox in camels which occurred in three different geographical regions of Iran; Tehran, Shiraz and Gorgan. The material from each area was kept separately at - 20°C until studied.

Cultural methods

About 3-5 g. of pox-infected material were ground in sterile and prechilled mortars with 10 ml. VM₃ containing penicillin and streptomycin at a final concentration of 1000 units/ml. of each.

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After centrifugation at 4000 r. p. m. for 10 minutes the supernatant was collected and frozen at -20°C . The following day the frozen material was thawed at room temperature and centrifuged at 4000 r. p. m. for 10 minutes; the supernatant was divided into three parts and then used as follows:

One part was kept undiluted, the second part diluted $1/5$ and the last part diluted $1/10$ using VM_3 as a diluent. One ml. of each part was then inoculated into each of 5 tubes of primary LK and Vero cells. After adsorption for 4 hours, the inoculum was discarded and replaced by 2 ml. of fresh VM_3 . The infected cultures together with non-infected control tubes were incubated at 37°C for 10 days.

Cultures showing distinct cytopathogenic effect (CPE) on the 8 to 10th day were frozen and used for further in vitro passages.

From the second passage, slight CPE appeared as early as 48 hours and was complete at 96–120 hours post-inoculation (Figs. 1 and 2).

Microscopic examination of the infected cells fixed in Bouin's fluid and stained by haematoxylin-eosin revealed inclusion bodies in cytoplasm and nucleus.

Each of the three strains has undergone 10 consecutive passages on LK and Vero cells. Wet and lyophilized samples of each passage have been stored at -70°C and -20°C , respectively.

Titration of Virus

Serial tenfold dilutions of the 10th passage of each strain grown on LK cells were separately prepared in VM_3 and inoculated in 0.2 ml. amounts in each of six culture tubes. After an observation period of 10 days, the 50% tissue culture infective dose (TCID_{50}) was calculated according to the method of Reed and Muench (9).

Host specificity of virus

One-humped young camels together with a suitable number of cattle, sheep, goats, rabbits, guinea-pigs, mice, rats and hamsters have been inoculated with either original or tissue culture adapted CPV, Teheran strain. After 12 days, the sheep and goats inoculated were challenged with 10,000 reaction doses of virulent homologous viruses.

To check the propagation of virus in different cell culture systems, a wide variety of mammalian cells were infected with each of the three isolates.

Ovoculture

Nine day old chick embryos were inoculated with 0.5 ml. of tissue culture adapted CPV (Teheran strain) which was diluted $1/100$. After inoculation they

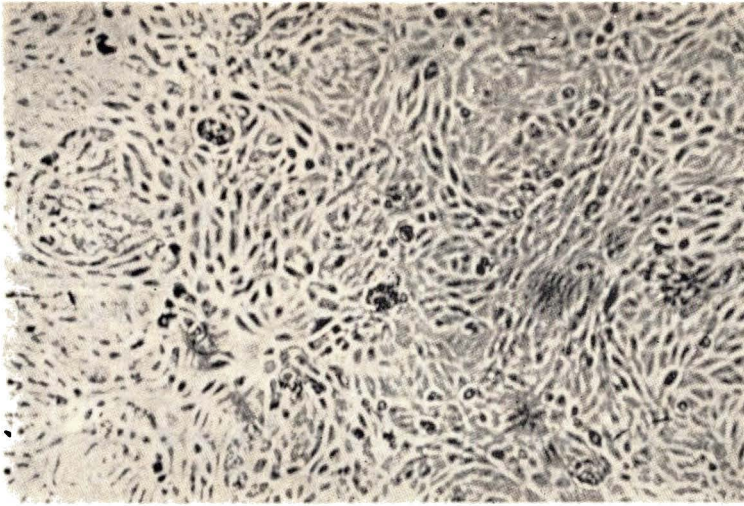


Fig. 1. Normal lamb kidney cells

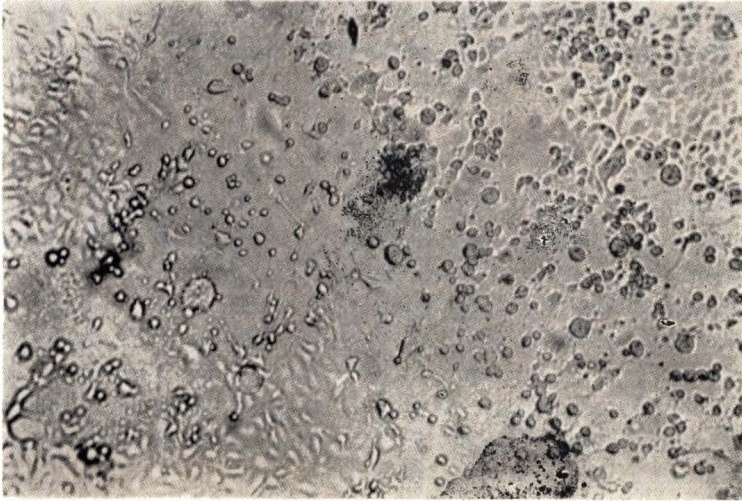


Fig. 2. Lamb kidney cells 96 hours after infection with camel pox virus

were incubated at 37°C and candled daily. At the end of 7 days, eggs were chilled and the chorioallantoic membrane harvested in Petri dishes.

Sensitivity to lipid solvents

Tissue culture CPV, Teheran strain, was mixed with diethyl ether (20% by volume) and the mixture was held at 4°C overnight. After the ether had been allowed to evaporate, the virus was titrated on LK cells.

0.05 ml. of chloroform was added to 1 ml. of tissue culture CPV, shaken for 10 minutes at 4°C, centrifuged for 5 minutes at 400 r. p. m. and the supernatant used for virus titration.

Heat stability

Five ml. of tissue culture propagated virus, Teheran strain, were placed in each of 5 test tubes and then heated to 56°C for 5, 10, 15 and 20 minutes. To check the infectivity, ten-fold dilutions of the heated materials were separately prepared and inoculated into each of 6 primary LK cell cultures.

PH sensitivity

VM₃ was dispensed in 12.6 ml. amounts in each of 15 prescription type bottles. The PH was adjusted from 3 to 10 by adding 1/10 normal HCl or 1/10 normal NaOH. To each bottle, 1.4 ml. of tissue culture CPV, Teheran strain, which had a titre up to 10^{-6.3}/ml. was added. The medium and virus were mixed well. After keeping for one hour at 4°C, 2 ml. of the material in each range was inoculated into each of 5 primary LK monolayers. The infected cultures together with 6 controls were regularly checked for appearance of CPE for up to 10 days.

Morphology

Passage 8 of tissue culture adapted Teheran strain was frozen, thawed and subjected to centrifugation at 1000 r.p.m. for 10 minutes. The supernatant was removed and centrifuged again at 40,000 r. p. m. for 2 hours in a Spinco ultracentrifuge, model L 50. The supernatant was then discarded and the sediment suspended in 0.5 ml. of physiological saline mixed with 2% phosphotungstic acid. A small drop of the mixture was then placed on film supports. After all the excess fluid had been removed with a fine capillary pipette, the films were dried and examined at the Faculty of Agriculture, University of Teheran under an electron microscope, Siemens L Minioscope I A.

A sample of the same virus was sent to the Institute for Microbiology and Infectious Diseases of Animals, University of Munich, Germany for further morphological observations.

Results

The highest titre of CPV inoculated in LK cell cultures was obtained at 96-120 hours, by which time the cells were completely degenerated. The titre then dropped gradually and after 240 hours of incubation the virus was not infective when inoculated into LK or Vero cell monolayers (Fig. 3).

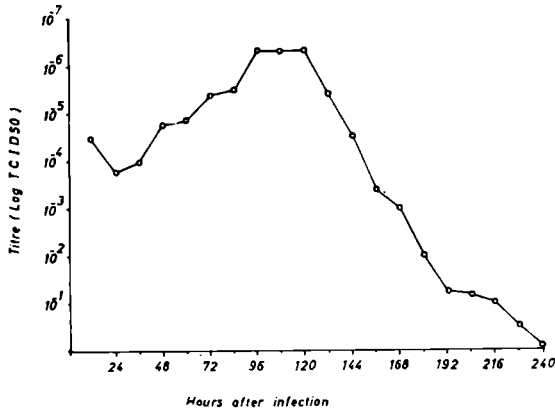


Fig. 3. Growth curve of Camel Pox Virus

As shown in Table 1 tissue culture adapted CPV, Teheran strain, caused generalized pox and death when inoculated in young camels 9 to 10 months old. Attempts to infect cattle, sheep, goats, and small laboratory animals were negative. CPV inoculated in sheep and goats did not induce any degree of immunity against a challenge dose of sheep and goat pox viruses.

Table 1
Host specificity of CPV

Animal species	Nr. of animals inoculated	Age	Route of inoculation	Amount inoculated	Local or general reactions
Camel	4	9 - 10 months	I / D	1 ml	Generalized pox
Cattle	6	6 "	"	6 ml	-
Sheep	6	6 "	"	4 ml	-
Goat	6	6 "	"	4 ml	-
Rabbit	8	Adult	"	2 ml	-
Guinea-pig	8	"	"	2 ml	-
Mice	10	"	"	1 ml	-
"	10	5 day-old	"	0.5 ml	-
Rat	6	Adult	"	1 ml	-
Hamster	8	"	"	1 ml	-

I/D: intradermal
- : no reaction

Clear CPE was observed in lamb kidney, lamb testis, calf kidney, calf testis, camel kidney, pig kidney, BHK-21, MS and Vero cells after infection with each of the three isolates.

Inoculation of the Teheran strain on the chorioallantoic membrane of 9 day old egg embryos resulted in the appearance of several distinctive pocks similar to those caused by vaccinia virus.

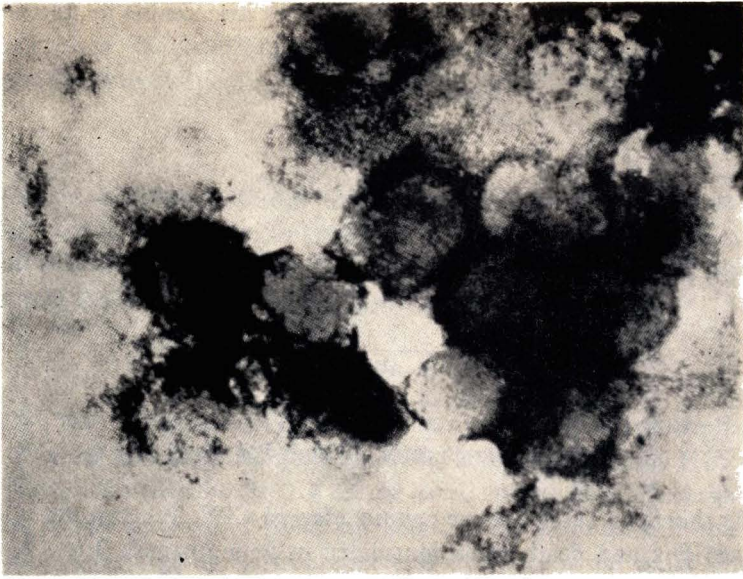


Fig. 4. Electron micrograph of camel pox virus, 72,000 ×

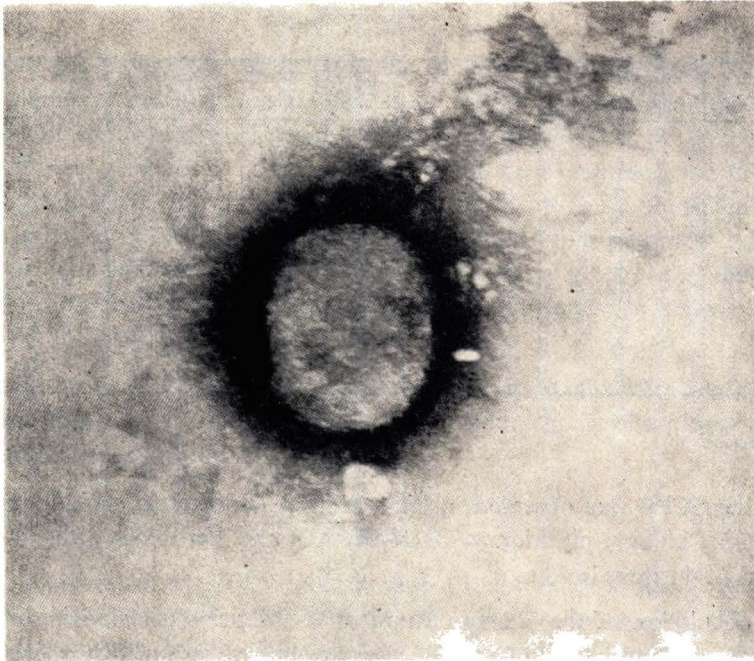


Fig. 5. Electron micrograph of camel pox virus, 108,000 ×

Our experiments showed that CPV is sensitive to both ether and chloroform, exhibiting a decrease in titre of more than 4 logs.

CPV heated for 5 and 10 minutes showed a reduction in titre of more than 2 and 4 logs, respectively. The virus heated at 56°C for 15 and 20 minutes appeared to be completely inactivated.

CPE did not appear in inoculated cultures which had PH ranges from 3 to 4.5. PH ranges 9 to 10 caused cell degeneration within 24 hours which were not related to the virus. The optimum PH varied between 6.8 and 7.4.

From the morphological point of view, the electron micrographs showed that the CPV is closely related to other pox viruses and has a diameter of approximately 270 m μ (Figs. 4-5).

Discussion

Camel pox is of economic importance in many parts of Africa and Asia.

Amanschulow et al. (1) considered that camel pox is identical to sheep pox and according to their observations camel pox can be transmitted to sheep. Borisovich et al. (3) reported that sheep pox hyperimmune serum was not effective against camel pox.

Several field investigations carried out in Iran proved that camels living with sheep heavily infected with sheep pox do not show any symptoms of pox and vice versa. Also in our laboratory we have failed to infect sheep by inoculation of camel pox virus. These animals remained susceptible to the challenge strain of homologous virus.

From these results it appears that the causative agents of camel pox and sheep pox are antigenically different and to prevent camels from infection, immunization should be carried out by a vaccine produced with a homologous virus.

Summary

Three isolates of camel pox virus were studied and adapted to chick embryos and tissue cultures originating from different animal species.

The highest yields of virus were obtained at 96-120 hours after inoculation of cell cultures.

Cattle, sheep, goats and small laboratory animals did not become infected by intradermal inoculation of CPV.

The virus is sensitive to lipid solvents and heat. It does not grow on cell cultures when the PH is below 4.5. The optimum PH for virus multiplication appears to be between 6.8 and 7.4.

Electron microscopy of CPV reveals ovoid particles which are approximately 270 m μ in diameter.

Résumé

Isolement, culture et caractérisation du virus variolique du chameau

Le virus de la variole du chameau a été isolé à trois reprises. On l'adapte sur embryons de poulets et sur cultures de cellules de différentes espèces animales. On obtient le meilleur rendement en virus 96 à 120 heures après infection des cellules.

On ne parvient pas à infecter les bovins, les moutons, les chèvres et les petits animaux de laboratoire avec le virus variolique du chameau. Le virus est sensible aux dissolvants des corps gras et à la chaleur. En cultures de cellules, il ne se multiplie pas à un PH en dessous de 4,5. La multiplication optimale s'effectue aux PH de 6,8 à 7,4.

L'examen au microscope électronique révèle des particules ovoïdes, d'environ 270 m μ de diamètre.

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