Arch. Inst. Razi, 1978,30,107-115

MORPHOLOGY AND PHYSICAL CHARACTERISTICS OF SHEEP AND GOAT POX VIRUSES

BY:

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INTRODUCTION

Poxviruses are the largest known animal viruses, their structure and composition are complex as compared to other viruses.

Poxviruses include several subgroups which affect human and many species of animals, although in many aspects these viruses are closely related, but they have different biological characteristics and have distinct neutralizing antigens (7,15).

Most of the reports on the morphological structure and physicochemical composition of poxviruses are based on the studies performed on Vaccinia Virus (5,6,8,14), and ORF virus (10).

In spite of the long time presence of sheeppox and goatpox in many countries of the world (12) particularly in Iran, information with regard to the physico-chemical characteristics of the causative agents are very limited (2), in this report the results of a comparative study carried out on Sheep pox and Goat pox viruses with regard to their morphology and physical properties are presented.

MATERIALS and METHODS

Virus:

Sheep pox virus strain RM / 65 at 32th passages and Goat pox virus strain Gorgan at 35th passages were used in this study, the viruses had been originated from naturally infected animals and attenuated by serial passages in primary lamb kidney cells (11).

At early passages, these viruses caused sever reactions when inoculated into susceptible animals, but lost their virulence upon subsequent passages.

Cell:

primary lamb kidney cells produced by trypsinization of aseptically removed kidneys from healthy animals and suspended in Hanks BSS containing 0.5 percent Lactalbumin and 10 percent calf serum and Antibiotics, the growth media distributed in 160 ml amount into two litres Povitsky bottles and incubated at 37°C, after formation of complete monolayer which usually took 4 to 6 days, the cells were used for virus infection.

Virus Production:

Fifteen complete monolayer bottles of primary lamb kidney cells were infected with each virus at multiplicity of infection of 5 TCID/cell, the viruses allowed to adsorb for 2 hrs. at 37°C, after the adsorption period, maintenance media was added and the infected cells were incubated at 37° C.

After 5 to 6 days when 100% CPE was observed the infected cells were scraped off the bottles with a rubber policeman and suspended in 4 ml of saline, the cells were homogenized in a Sorvall homogenizer for 1 min. and then centrifuged at 5000 rpm for 10 min, in a IEC centrifuge to sediment cell debris, the pellet was discarded and the supernatant was saved for virus purification.

Sucrose Density Gradient Centrifugation:

Linear gradient of 70 - 30% Sucrose in Tris buffer PH 7.4 was made in 5 ml Nitrocellulose tubes with gradient maker, one ml of crude virus suspension was layered on the top of the gradient and the tubes were centrifuged at 22000 rpm for 90 min. in a Spinco ultracentrifuge using a SW39 swinging bucket, the virus band was collected by puncturing the side of the tube with a needle connected to a syringe and the collected virus was then dialysed against V.S. 0.1 M phosphate buffer PH 7.2.

Cscl Density Gradient Centrifugation:

Linear Cscl gradient with density of 1.4 to 1.1. in Tris buffer PH 7.4 was prepared in 5 ml tubes and one ml Sucrose purified virus suspension was layered on the top of the gradient, the tubes were centrifuged at 30000 rpm for 4 hrs. as described above.

Ten drops fractions were collected from the bottom of the tube by piercing the tube using an ISCO fraction collector, the tubes were sealed immediately and stored at 4 C for density measurement.

Infectivity and density measurement:

The density of fractions were calculated by weighing 50 microlitre of Cscl fractions with an analytical balance.

The infectivity of each fraction was determined by diluting each fraction 1/1000 in VM3, a serial 10 fold dilutions were prepared and 0.1 ml of each dilution was inoculated in each of 5 tubes containing monolayer of primary lamb kidney cells, the tubes were examined daily for the appearance of CPE, the titer of the virus was calculated according to the Reed and Muench method (13).

Electron Microscopy:

The virus band obtained after density gradient centrifugation was dialysed against 0.5 M ammonium acetate PH 7.2, a sample was placed on Formvar coated grides and negatively stained with 3% Sodium phosphotungstate, the grides were examined in a Phillips E M 400 Electron Microscope at 80 K V.

RESULTS

Both Sheep pox and Goat pox viruses could be grown on primary lamb kidney cells with good yield of intracellular virus particles (judged by virus purification), both type of viruses could be purified by Sucrose gradient centrifugation, but viral band obtained by this method still contained some impurities, for further purification the method of Cscl density gradient centrifugation was used, both viruses appeared as a sharp band in the middle of the tube, density measurement of the virus band in Cscl revealed slight difference between the density of Goat pox and sheep pox viruses, Goat pox virus banded at a region with density of 1250 but sheep pox at a density of 1258/ ml.

Fig. 1 shows the density of the two viruses with their infectivity peaks, it is also shown that the infectivity titer of each virus corresponds to the single sharp band after Cscl density gradient centrifugation, the purity of the viruses was examined by Electron Microscope.

Fig. 2 shows a preparation of sheep pox virus which was quite pure and free from cellular debris.

In order to estimate and compare the size of Sheep pox and Goat pox viruses, a large number of each virus on the photographs were measured and the average size was estimated (table 1).

Virus	No. of particle measures	Average size in nm	
		Lenght	Width
SPV	28	320	280
GPV	33	260	235

Table 1 - the average size of sheep pox and Goat pox viruses.

Both viruses were brick shape with an average lenght of 320 and 260 nm for sheep pox and Goat pox viruses respectively.

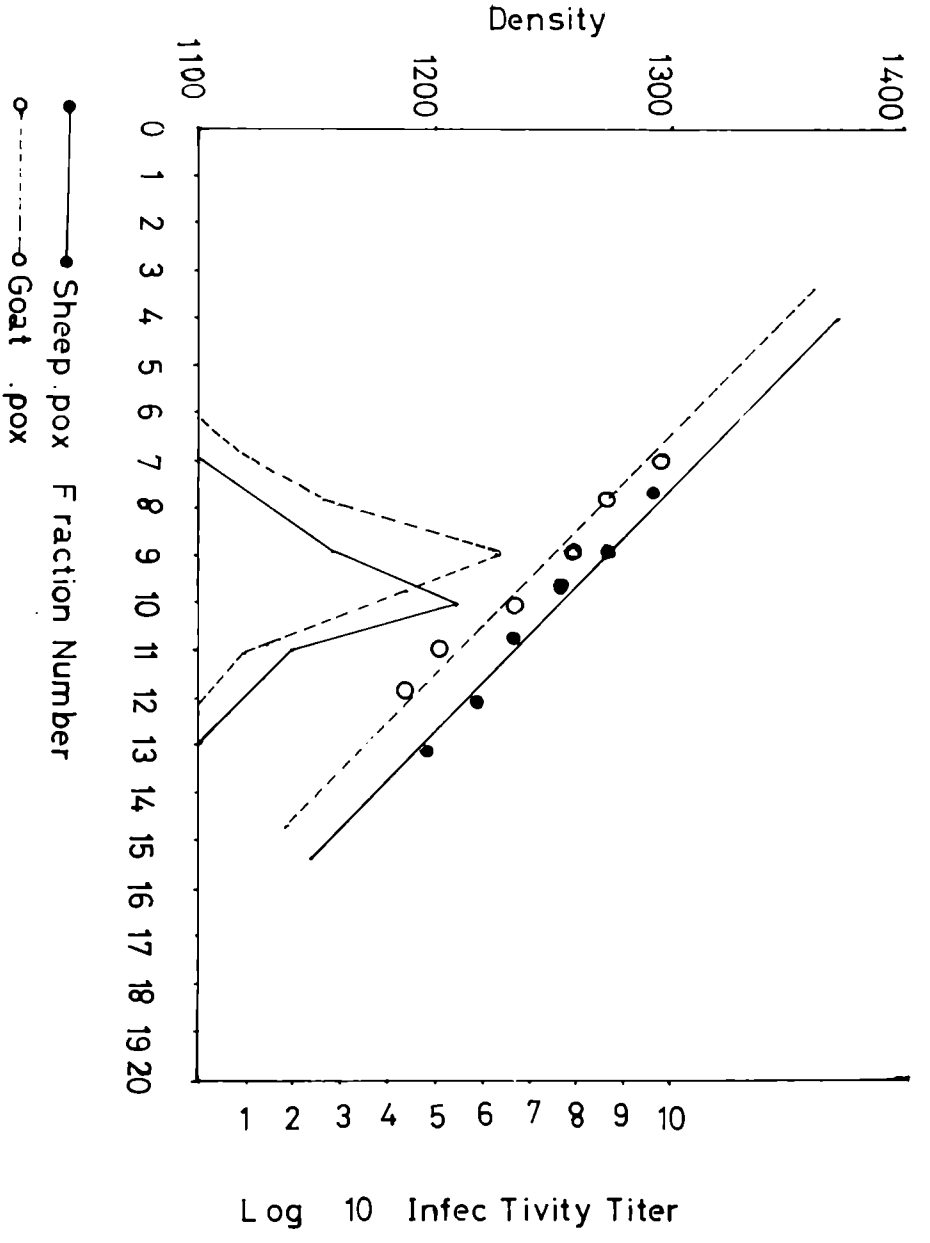

Fig. 1- Sheep pox and Goat pox viruses were prepared and centrifuged in Cscl as described in material and methods, fractions were collected and their density and infectivity was determined.

Morphologically both viruses were similar in appearance with tubular like structures on the surface (Fig. 3, 4), in addition some virus particles appeared with smooth surface and a membrane like structure surrounding the virus particles, these particles are known as C-Type and were abundant in the prepartion of both sheep pox and Goat pox viruses (Fig. 5,6).

DISCUSSION

Studies reported on the morphologhy and structure of poxviruses are numerous (1,3,9,10), these studies show that there are some differences between the morphological structure of some subtypes of poxviruses (10), in this study morpho logically both sheep pox and Goat pox viruses resembled some other members of poxvirus group (4).

Although purification of the two viruses was carried out under the same conditions but there was slight difference between the density of the viruses, it is possible that the proportion of nucleic acid and protein is slightly different in the two viruses examined, in addition size measurerment of a large number of virus particles showed that sheep pox virus particles were slightly larger then the Goat pox viruses (60 nm) .

The size of sheep pox and Goat pox viruses was close to the size of variola vaccinia subgroup of poxviruses (7), it should be mentioned that intracytoplasmic viruses were quite heterogenous in size and for this reason to minimize the error, a large number of virus particles were measured.

Presence of C-Type particles in poxvirus preparations is commom (4), it is believed that these particles are produced during the negative staining preparation, and the phenomenon occurs during the drying period of the grides.

Cohen et al. (2) have studied morphological replication of sheep pox virus in lamb kidney cells and showed that it was similar to other members of the group, however there is no clear report on the morphological characteristics of Goat pox virus, because of the routine production and application of Goat pox and sheep pox vaccine, this study was carried out to obtain some informations about the properties of the above viruses.

SUMMARY

Morpological characteristics of sheep pox and Goat pox viruses were studied, although these viruses are antigenically related but their physical properties are slightly different.

Purification of sheep pox and Goat pox viruses by sucrose and Cscl and then Electron Microscope examinations showed that the size of sheep pox virus is larger than Goat pox, density of these viruses are slightly different.

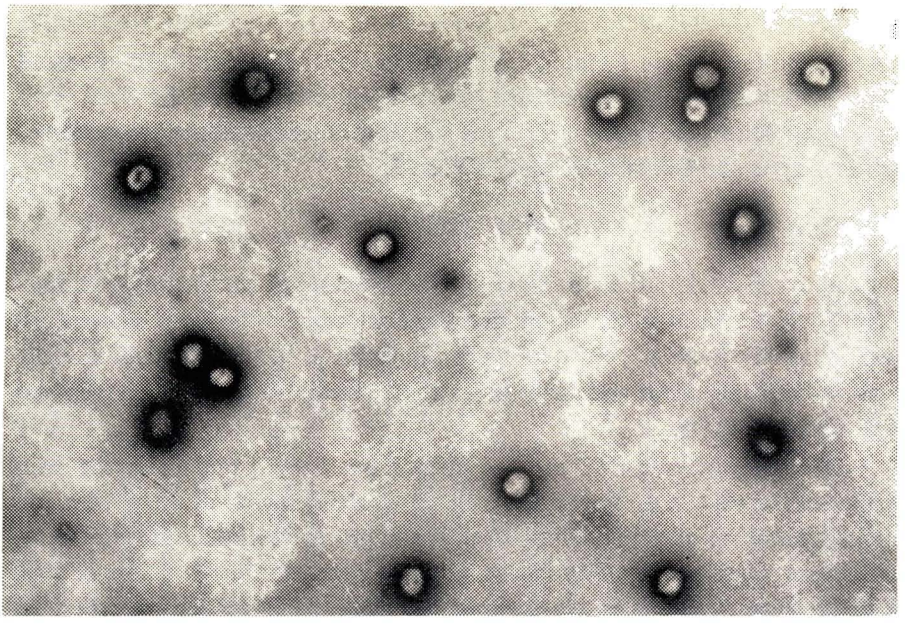

Fig. 2- Preparation of Sheep pox virus purified by Cscl Density Gradient Centrifugation showing virus particles free from cellular debris. X 15000

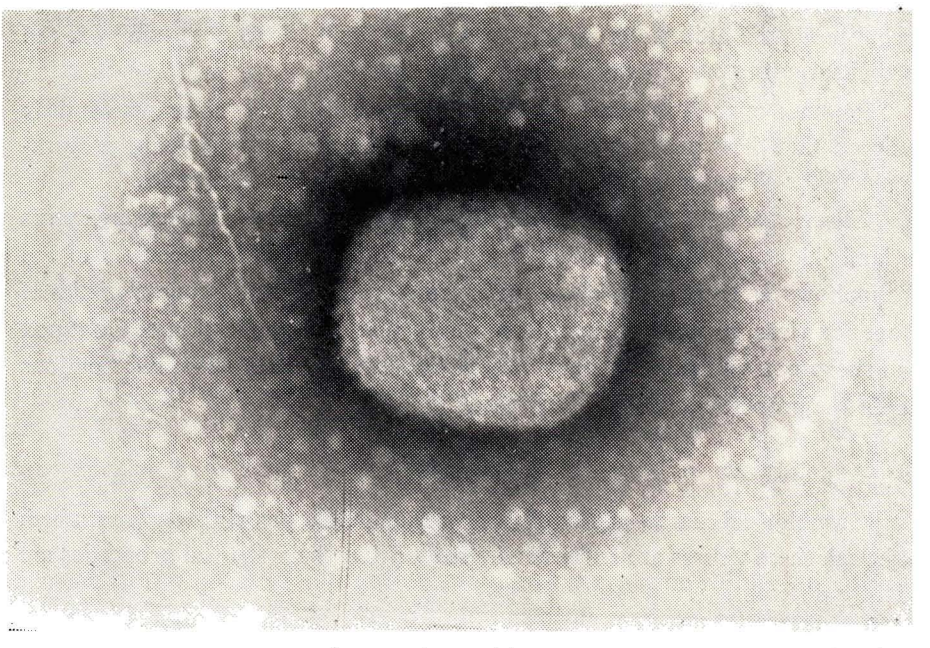

Fig. 3- Sheep pox virus negatively stained with sodium phosphotungstate showing tubular structures on the surface. X 180000

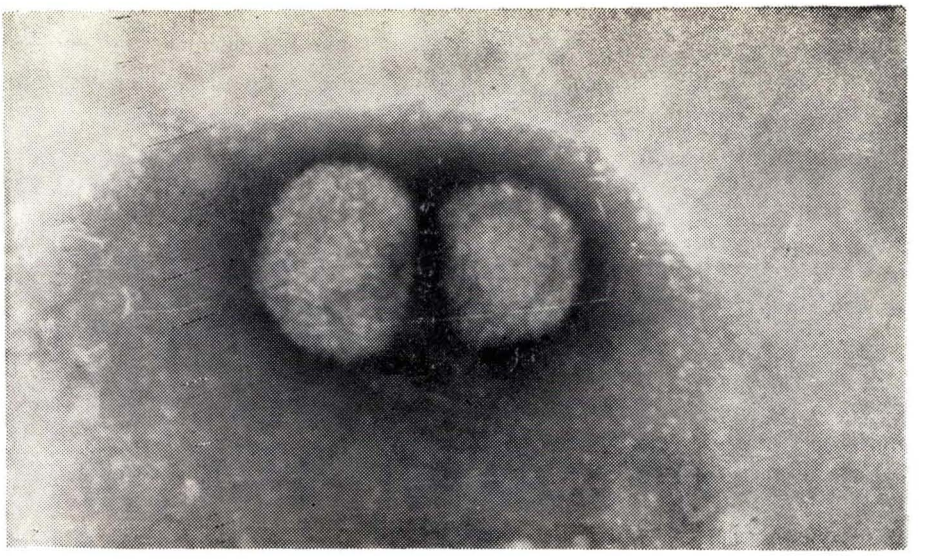

Fig. 4- Goat pox virus prepared as in Fig. 3. x 180000

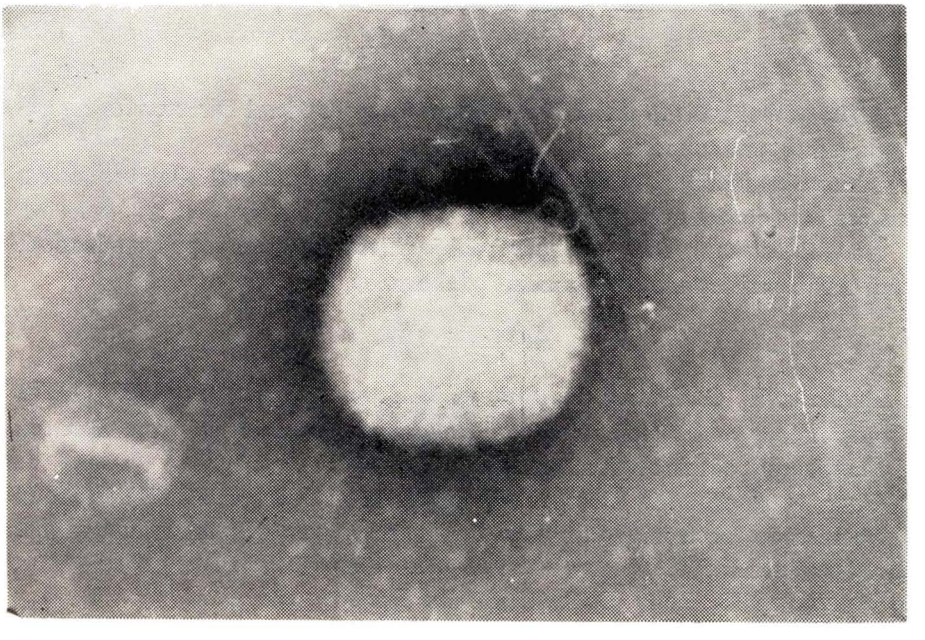

Fig. 5- C-Type particle in Sheep pox virus preparation showing an outer membrane. x 200000

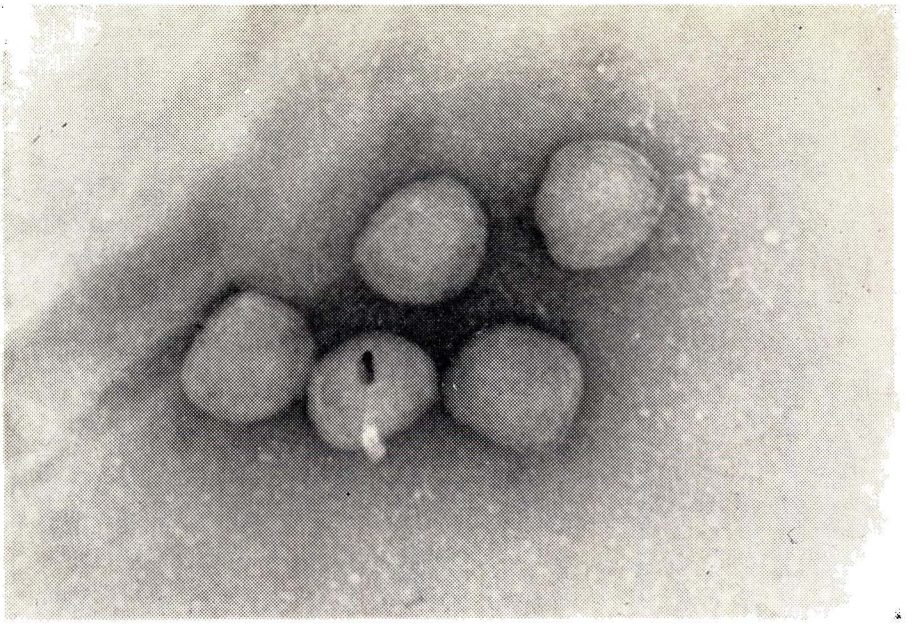

Fig. 6- Several C- Type particles in Goat pox virus preparation. X 130000

ACKNOWLEDGEMENTS

The author wish to thank Dr. H.Ramyar, the General Director for his encouragement and support.

I am also indebted to Dr. M. Hessami for the subject and whatever success has been achieved in this paper.

I wish to thank Dr. M.S.Shahrabadi for Electron Microscope examinations and valuable advice and proof reading of the manuscript.

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