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# Characteristics of a Kenyan camelpox virus

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SUMMARY

A virus was isolated from pock-like vesicular eruptions of camels in Northern Kenya. This virus was shown to be a pox virus with many characteristics of members of the Orthopox group. It appears to be identical with camelpox strains from Iran and has similar properties to certain East African variola strains.

## INTRODUCTION

For many years outbreaks of camelpox have been reported from the camel-raising areas of Kenya. Mortality appears confined to the younger animals. Older animals suffer some loss of condition and a fall in milk production and weight. Deaths from intercurrent disease, notably trypanosomiasis, increase in affected herds.

Some interest has been shown in the close relation between variola strains from East Africa and camelpox virus (Baxby, 1972).

This communication records some of the characteristics of a Kenyan camelpox strain, the first to be isolated in East Africa.

## MATERIALS AND METHODS

### *Viruses used*

Two camelpox strains H 520 and M 115, were found to be identical and H 520 was used in this work. A sheeppox and a goatpox strain from a mixed flock of sheep and goats were designated O 180 and F 53 respectively. A vaccinia virus (Elstree) was obtained from the Kenya Medical Research Centre. Cowpox was a Kenyan strain of unknown history, lumpy skin disease virus (LSD) was the L 2490 strain from Kenya, fowlpox was the Beaudette attenuated strain and orf virus was the Kenya vaccine strain.

### *Egg inoculation*

Titrations and culture on the chorioallantoic membrane (CAM) of embryonated eggs were carried out by standard methods and the eggs were incubated at 34·5, 37, and 39° C. Twelve-day embryos were used.

### *Tissue culture*

BHK 21/Clone 13 cells (Macpherson & Stoker, 1962) and Vero cells were grown and maintained according to methods already described (Davies & Lund, 1974).

Pre-pubertal merino lamb testis and lamb kidney cells, and calf kidney cells were prepared largely following the methods of Prydie & Coakley (1959). Petri dishes were seeded for plaque studies with 150,000 BHK cells per ml., and 200,000 cells per ml. of the other cell types. They were overlaid with a 1 % mixture of carboxymethylcellulose in the appropriate medium, and stained after five days with 1 % crystal violet in 20 % methyl alcohol.

*Staining methods*

Flying cover-slip preparations of tissue culture cells and the CAM were fixed and stained by standard histological techniques.

*Haemadsorption*

Cultures infected with virus, together with uninoculated control cultures, were washed three times in phosphate buffered saline (PBS) and a 0·5% suspension of cockerel erythrocytes in Earle's medium was added to each tube. The tubes were kept at 37° C. for 30 min. and then examined.

*Haemagglutination*

This was carried out using standard methods with cockerel erythrocytes, which were shown to haemagglutinate with vaccinia virus.

*Fluorescent antibody methods*

The indirect method was used with sera prepared in rabbits against the various viruses. Anti-species conjugates were prepared by methods already described (Davies & Lund, 1974).

*Ether and chloroform sensitivity*

Control and ether- or chloroform-treated virus suspensions were kept at 4° C. for 18 hr. with intermittent shaking.

*Preparation of antisera*

New Zealand white rabbits of 3–4 kg. were inoculated with tissue culture harvests of virus together with an equal volume of Freund's incomplete adjuvant, 5 ml. per rabbit. This was inoculated in multiple intradermal, subcutaneous and intramuscular sites, and repeated after three weeks. The rabbits were bled out after a further 3 weeks.

*Pathogenicity studies*

Pathogenicity studies were carried out in infant mice by intracerebral inoculation, CAM inoculation of embryonated eggs and intradermal inoculation of rabbits and sheep. Intrafollicular inoculation of 3-day-old chicks was carried out by removing secondary wing feathers and introducing the virus suspension into each follicle with a fine needle.

## RESULTS

### *Tissue culture*

Primary inoculation of material from the camel lesions (H 520) produced cytopathic effects in BHK cells, Vero cells, lamb testis and lamb kidney and calf kidney cells. Foci of rounded cells first appeared, these enlarged and at the centre of the cytopathic plaque cells freed themselves from the glass. Secondary plaques then formed, the cytopathic changes progressively involved the whole of the monolayer and the cells detached after rounding. The effects were similar in all the cell types studied.

Flying cover-slips stained with haematoxylin and eosin from cultures infected with H 520 showed intra-cytoplasmic eosinophilic inclusions in foci which could be related to the changes seen with the light microscope. The inclusions stained green with acridine orange and red with the Feulgen reaction. The lumpy skin disease virus used as a control DNA virus stained in a similar manner. Rift Valley Fever, an RNA virus, stained orange red with acridine orange.

The H 520 virus produced plaques in BHK cell petri dishes, in Vero cell cultures and lamb testis and calf kidney cells. The plaques formed remained small, from 0·4 to 0·8 mm. diameter after 5 days and were irregularly round in outline.

Cultures of H 520 virus showed haemadsorption compared with uninoculated control cultures. Tissue culture harvests of these cultures were used in haemagglutination tests together with vaccinia. The H 520 strain haemagglutinated poorly at 1/8; vaccinia haemagglutinated the red cells used at a 1/64 dilution.

The H 520 virus was sensitive to pH in the range 3–5 and again between pH 8·5 and pH 10.

### *Egg culture*

H 520 produced pocks on the CAM on primary isolation from camel material. Incubation at 37° C. gave pocks of 0·5 to 1 mm. diameter. These were grey white and of a proliferative nature. At 34·5° C. the pocks were 0·2–0·4 mm. diameter and were slightly domed. No central haemorrhage or necrosis was seen. Histological sections of the CAM of embryos inoculated with H 520 gave the same cellular inclusions in the CAM that were seen in tissue culture. The eosinophilic intra-cytoplasmic inclusions and Feulgen positive reaction could be shown in the cells in the pocks. No pocks were formed at 39° C. A vaccinia virus used for control produced pocks at all three incubation temperatures, with differing pock morphology at the three temperatures.

### *Ether and chloroform sensitivity*

H 520 virus was not sensitive to ether but was sensitive to chloroform. The titre was reduced from $10^{4\cdot6}$ TCID50/0·1 ml. to $10^{1\cdot8}$ TCID50/0·1 ml.

### *Pathogenicity*

H 520 did not produce any reactions in rabbits or sheep after intradermal inoculation or after scarification. Vaccinia virus produced initial hyperaemia and oedema with necrosis at the inoculation and scarification sites.

Table 1. *Indirect fluorescent antibody tests carried out between various pox viruses and their antisera*

| | Antisera | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Viruses | H 520 | Vaccinia | Cowpox | O 180 | F 53 | L 2490 | Fowlpox | Orf |
| Camelpox (H 520) | + | + | + | − | − | − | − | − |
| Vaccinia | + | + | + | − | − | − | − | − |
| Cowpox | + | + | + | − | − | − | − | − |
| Kenyan sheeppox (O 180) | − | − | − | + | + | + | − | − |
| Kenyan goatpox (F 53) | − | − | − | + | + | + | − | − |
| Kenyan LSD (L 2490) | − | − | − | + | + | + | − | − |
| Fowlpox | − | − | − | − | − | − | + | − |
| Orf | − | − | − | − | − | − | − | + |

H 520 virus produced no reaction when inoculated into the defoliated feather follicles of day-old chicks.

H 520 showed very low pathogenicity for chick embryos, most embryos being alive at 5 days. Vaccinia had an LD50 of $> 10^6$ TCID50/0·1 ml. In infant mice the LD50 for H 520 was $10^{3·4}$ TCID50/0·02 ml. compared with a figure of $> 10^6$ for vaccinia.

*Fluorescent antibody tests*

A summary of the results of the series of indirect fluorescent antibody tests is given in Table 1. There was cross-fluorescence between virus and antisera of members of the Orthopox virus groups, which includes H 520 camelpox virus, vaccinia and cowpox. There was no cross-fluorescence between these viruses and the Ungulopox group, the sheep- and goatpox viruses and the lumpy skin disease virus. These viruses did, however, cross-fluoresce with their group antisera. No cross-reaction was seen with the fowlpox virus, or with orf virus.

## DISCUSSION

The camelpox virus strains from Iran and from Russia have been shown to be closely related if not identical (Marennikova, Shenkman, Shelukhina & Maltsova, 1974). The Iranian camelpox virus has been classified as an Orthopox virus (Mahnel & Bartenbach, 1973; Baxby, 1972). The results of this work with Kenyan camelpox virus show that it is probably identical with the Iranian and Russian camelpox viruses (Ramyar & Hessami, 1972; Baxby, 1972; Marennikova *et al.* 1974). Their significance, in addition to the economic losses sustained by camel owners, is in the close relation which exists between camelpox virus and various strains of variola from East Africa (Bedson, Dumbell & Thomas, 1963; Baxby, 1972). Differentiation between camelpox and variola has been made by the

demonstration of minor antigenic differences (Gispen & Brand-Saathof, 1974) and by the characteristics of the cytopathic effect in tissue culture (Baxby, 1975).

Reports from the recent field outbreaks of camelpox in Northern Kenya indicate that people drinking milk from affected animals developed ulcers on the lips and in the mouth. It has not been possible to verify this observation. The relation between variola and camelpox in Kenya, and the pathogenicity of this virus for humans, remain to be resolved.

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