

## **Characteristics of a virus causing a pox disease in sheep and goats in Kenya, with observations on the epidemiology and control**

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### SUMMARY

The sheep and goat pox viruses isolated in Kenya are not host specific as in the Middle East and India. The virus strains from one species are of a similar pathogenicity for the other, and the same virus appears to occur in field outbreaks in mixed flocks. A silent infection with no skin lesions occurs in the field outbreaks.

The virus grows well in lamb testis cell cultures, it does not haemadsorb nor haemagglutinate and is ether sensitive. Most strains could not readily be adapted to grow on the chorioallantoic membrane of embryonated eggs; one isolation only was made in this way. Staining of the virus inclusions showed that they were of DNA; the virus resembled that of lumpy skin disease in many characteristics.

It was possible to cross-protect both species with virus of sheep or goat origin. A vaccine was made from tissue culture virus adsorbed onto aluminium hydroxide gel and treated with formalin.

### INTRODUCTION

Sheep and goat pox viruses have been extensively studied in the Middle East and India, where under natural conditions the viruses are host specific (Balozet, 1931; Bennet, Horgan & Heseeb, 1944; Lall, Singh & Singh, 1947; Rafyi & Ramyar 1959; Murty & Singh, 1971; Sharma & Dhanda, 1972). Vaccines which are used against these diseases are those of the Borrel type made with virus of animal origin (Rafyi & Mirchamsy, 1956) or with virus of tissue culture origin (Ramyar, 1965). These have been used as formalinized and non-formalinized preparations. Attenuation of the sheep pox virus has been successfully achieved by Agyun (1955), Ramyar & Hessami (1967) and Martin, Ergin & Koylu (1973).

Some work has been carried out in Kenya on the pathology of the skin lesions (Plowright, MacLeod & Ferris, 1959) and on the growth of sheep pox virus in tissue culture (Plowright & Ferris, 1958; Coakley & Capstick, 1961). Field observations of the Kenyan outbreaks has revealed a major difference from those recorded in the Middle East and India. The mixed flocks of sheep and goats kept in the range areas were affected simultaneously and with comparable pathogenicity by a pox-like disease. This was generally of low mortality and a morbidity of up to 60%. Primary reports of disease have invariably been from the northern or southern parts of the nomadic pastoral areas (National Atlas of Kenya 1970), or

have followed movements of animals from these areas. These are in ecological zones IV, V and VI (Pratt, Greenway & Gwynne, 1966) which are low rainfall areas of low agricultural potential.

The extent of the outbreaks demanded that some vaccine be used in an effort to control it. A vaccine was prepared from indigenous strains which were grown in tissue culture, adsorbed onto aluminium hydroxide gel and treated with formalin. The method for its preparation and its use are described.

## MATERIALS AND METHODS

### *Virus characterization*

#### *Virus isolation*

The tissue samples were minced with sterile scissors and ground in a pestle and mortar with sterile sand. They were then frozen and thawed quickly with dry ice and alcohol three times. The tissues were inoculated with 500 i.u. penicillin and 500  $\mu$ g. of streptomycin sulphate per ml. for 1 hr. at room temperature, and made up to a 10% suspension in phosphate buffered saline. The suspensions were centrifuged at 2000 rev./min. for 10 min. and 0.1 ml. amounts of supernatant fluids inoculated into either lamb testis or BHK 21/C13 cell cultures, by methods already described (Prydie & Coakley, 1959; Davies, Krauss & Lund, 1972).

#### *Virus identification*

This was made by standard haematoxylin and eosin (H & E) staining of cover-slip preparations to demonstrate eosinophilic pox inclusion bodies, and by the direct or indirect fluorescent antibody techniques (Davies *et al.* 1972) as used for lumpy skin disease.

#### *Virus strains*

The sheep pox virus strain was from an outbreak in a mixed flock, this was designated O 180. A virus strain from a goat in the same flock was designated F 53, and these were used in this study.

#### *Fluorescent antibody methods (FAT)*

*Direct.* Antisera for conjugation were prepared in two ways.

(a) By inoculating rabbits of 2–3 kg. with a mixture of Kenya sheep/goat pox virus and incomplete Freund's adjuvant. Three inoculations of an infected tissue culture harvest with an equal volume of adjuvant to make 5 ml. were given at multiple subcutaneous, intradermal and intramuscular sites and intraperitoneally, at 3 week intervals.

(b) Sheep recovering from experimental pox infection were reinoculated with virus and Freund's adjuvant and serum was harvested 3 weeks later. If this could be shown to be free from antibody to the common sheep virus diseases in Kenya, it was used for conjugation.

The precipitation of gamma globulins and conjugation with fluorescein isothiocyanate was by methods already described (Davies & Lund, 1974).

*Indirect.* Anti-ovine and anti-rabbit gamma globulins were prepared in rabbits and cockerels respectively and conjugated with fluorescein isothiocyanate by methods already described (Davies & Lund, 1974).

Sera were screened for antibody to pox virus by an indirect method with sera inactivated at 56° C. for 30 min. and diluted 1/10 in phosphate buffered saline. These were applied together with positive and negative control sera to acetone fixed coverslip preparations of lamb testis or BHK cells with 15–40 % of the cells showing specific fluorescence to sheep/goat pox virus. Incubation was for 40 min. at 37° C. when the cover-slips were washed in three changes of PBS. The conjugated sheep anti-globulin was then applied for 30 min. and, after further washing, the cover-slips were mounted in buffered glycerol.

Further characterization techniques were as described by Davies, Mungai & Shaw (1975).

#### *Vaccine preparation*

##### *Virus strains*

Strains of F 90, a goat strain, and I 306, a sheep strain, were used in the cross protection and pathogenicity experiment, and a freeze dried pool of two goat and two sheep strains was used for the preparation of vaccine for field use. The virus was used at the third to the fifth passage. No alterations in the virulence of the virus have occurred at this stage (Plowright *et al.* 1959; F. G. Davies, unpublished observations).

##### *Virus growth*

Growth characterizations of a Kenyan sheep pox virus strain, the Kedong strain, were studied by Coakley & Capstick (1961) who used it for the preparation of a lumpy skin disease vaccine. They showed the convenience of using a high multiplicity of virus for vaccine preparation. Virus was grown on monolayers prepared on 16 oz. medical flats. These were harvested when 60 % of the monolayers showed cytopathic effects at 4–6 days after inoculation and the harvest was centrifuged at 3000 rev./min. for 15 min. to remove cell debris. This was then used to inoculate roller bottle cultures (CR 110 Flow Laboratories), 5 ml. being adsorbed for 1 hr. on the rollers and the cultures then washed twice by 5 min. rolling with changes of phosphate buffered saline (PBS). The maintenance medium was then added. Cultures were harvested by freezing and thawing three times when 60–70 % of the monolayers showed cytopathic changes. The harvest was centrifuged as before and 5 % fetal calf serum was added. Samples were taken for titration and for bacterial sterility tests, and the remainder stored at –20° C. The harvest was used for vaccine preparation if the titre was 10<sup>5.5</sup>/ml. or greater. It was diluted with phosphate buffered saline to give approximately 1000 TCID<sub>50</sub>/ml. of final suspension, and mixed with an equal volume of alhydrogel at 4° C. The virus, saline and alhydrogel mixture was stirred for 48 hr. at 4° C. Merthiolate was then added to a final dilution of 1/10,000 and also 0.03 % formalin. The vaccine was stored at 4° C. while safety testing was carried out.

*Safety testing*

The lamb testis tissue culture used for the preparation of the vaccine was examined for the presence of other viruses. Control cultures were maintained for 3 weeks and monolayers were grown on flying cover-slips and stained by means of direct fluorescent antibody techniques for bluetongue, Nairobi sheep disease and Rift Valley fever viruses. Four sheep were inoculated with 1 ml. of the vaccine subcutaneously, the sheep kept under observation for a 4 week period and the antibody response assayed. The pre- and post-inoculation sera were screened for antibody to the three sheep and goat virus diseases mentioned above.

*Sheep and goat virus titrations*

Corriedale sheep of 6 months to 1 year were obtained together with goats of a Masai type. They were screened against pox virus antibody by an indirect fluorescent antibody method. Virus was inoculated at four different sites per dilution on the shaved sides of each animal. The inoculations were made intradermally with 0.1 ml. volumes.

*Adjuvant*

A commercial preparation of 2% alhydrogel was used.

*Serum neutralization*

Tests were carried out by a constant-serum varying-virus method in lamb testis cells. The sera were inactivated at 56° C. for 30 min. and mixed with the appropriate virus dilution for 1 hr. at 37° C. and then overnight at -4° C. The tissue culture tubes were inoculated with 0.1 ml. amounts of virus-serum mixture and read 7 days after inoculation. The results were expressed as the differences between the pre- and post-inoculation titres obtained.

## RESULTS

*Field investigations*

Many outbreaks were visited to study the character of the infection. Some parts of the pastoral areas were clearly an enzootic situation for 82% of animals sampled showed specific antibody to the viruses. When disease occurred in these areas the morbidity was found to be 5-10% with a very low mortality; younger animals were generally affected. Elsewhere, no antibody to sheep and goat pox virus was found and extensive epizootics involving many thousands of animals were seen. Studies in these situations showed that while up to 60% of the animals showed skin lesions, the remainder of the animals in the flocks developed an antibody response to the viruses. The antibody titres were comparable with those from animals which had skin lesions. It is thought this indicates that a silent infection occurs in a significant proportion.

Most outbreaks have occurred in the indigenous hair sheep and goats and mortality rates have been consistently low, below 2%. Higher mortality has been

seen at times of drought when young lambs and kids seem more susceptible, and also in outbreaks in wool sheep.

Spread of the virus in the field was effected primarily by the habit of night herding when flocks are closely crowded together, as protection against predators. Inhalation is thought to be the main route of infection, for lung lesions are seen in most cases. The course of the disease in an affected flock is rapid, and lesions or seroconversion will be found in all animals in a particular flock within 4 weeks of the first case being observed. The continual movement of rams and fat sheep for trade, family and grazing reasons ensures the dissemination of virus from an infected focus. Further contact at water holes also allows of spread from flock to flock. Good control has been exerted by preventing any contact between flocks.

The range areas carry considerable numbers of game animals and the possibility that they were infected in these epizootics was considered. A total of 120 sera from Thomson's and Grant's gazelles, impala, wildebeest and kongoni were screened for antibody to these viruses. None was found. Fifty of the sera came from an area where there had recently been an outbreak of disease.

#### *Skin lesions*

These were found on both sheep and goats in the mixed flocks of indigenous hair sheep and Masai goats. Lesions were most frequently found on the hairless areas of the animals, notably the ears and around the mouth, under the tail and on the perineal areas, in axillary and groin areas and sometimes spreading along the abdomen. More rarely the whole animal was covered with lesions. The individual pocks were from 0.5 to 1.5 cm diameter, irregularly round and consisting of thickened areas of epidermis and dermis over which the hairs were erect, readily demarcating such areas from adjacent healthy skin. The pustule or vesicle formation associated with most pox viruses was not seen in this disease. Abrasion of the superficial layers of the epidermis to produce bleeding and scab formation was seen in approximately one lesion in ten. Thickened plaques were found in the oropharynx and in the alimentary tract, notably the abomasum, and lesions were regularly found in the lungs. These were depressed grey areas of 0.3–2 cm on the parietal surface.

#### *Virus isolation and characterization*

Many virus strains were isolated from the skin lesions of sheep and goats in the outbreaks.

The sheep and goat pox strains grew best in lamb testis cells and less well in lamb kidney; no cytopathic effects were seen on primary inoculation of calf kidney cultures or Vero cells. The virus did not generally produce cytopathic effects in BHK cells on primary isolation, but certain strains have done so. We suspect that this is due to the varying susceptibility of the cell line. The cytopathic effect of the sheep and goat pox strains was similar to that produced by lumpy skin disease virus. Small foci of cells show an increased density under the microscope with the cell outlines more clearly demarcated. A stranding of the cytoplasm occurs and

later rounding of the cells with detachment from the monolayer. Plaques were produced by both strains of virus in lamb testis cells.

Coverslips stained with haematoxylin and eosin showed intra-cytoplasmic eosinophilic inclusions in cell foci which could be related to the cytopathic changes observed with the microscope. By means of the fluorescent antibody tests, the antigen was generally seen as a round body comparable with the H & E stained inclusions. Other fine granular fluorescent particles were seen in the cytoplasm in addition to the large fluorescent areas. The inclusions stained green with acridine orange and red with the Feulgen reaction. A 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.

#### *Egg culture*

O 180 and F 53 viruses did not grow on the CAM on primary isolation from sheep and goat tissues. Six repeated passages did not produce pocks at any of the incubation temperatures. One strain from a goat has produced pocks on the CAM and then only on 10–20% of the inoculated eggs. The pocks were formed at 37° C. and at 34·5° C., were very small (0·3 mm.) and grey white. A lumpy skin disease virus produced small (1 mm) pocks after 4 passages at 37° C.

#### *Ether sensitivity*

Both O 180 and F 53 were sensitive to ether with  $10^{3.7}$  TCID<sub>50</sub> per 0·1 ml. and  $10^{3.4}$  TCID<sub>50</sub> per 0·1 ml. of virus inactivated respectively.

The virus strains did not show any haemadsorption or haemagglutination. They were not pathogenic for infant mice (LD<sub>50</sub> >  $10^4/0.02$  ml.), for chick follicles nor rabbits, where no reactions were seen. The sheep strain was pathogenic for goats and the goat strain for sheep.

#### *Fluorescent antibody tests*

A summary of the results of a series of indirect fluorescent antibody tests is given in Table 1 of a previous paper (Davies *et al.* 1975). There is fluorescence between viruses and antisera of the Orthopox virus group; which include camelpox virus, vaccinia and cowpox. There is no cross fluorescence between these viruses and the Ungulopox virus group O 180, F 53 and the lumpy skin disease virus. These viruses do however fluoresce with their group member antisera. No cross reaction was seen with the fowl pox virus, nor with orf virus.

#### *Vaccine studies*

##### *Cross protection*

The results of an experiment designed to show that there is cross immunity between sheep and goats vaccinated with different species isolates are summarized in Table 1.

There is apparently a complete cross protection of sheep vaccinated with goat virus and of goats vaccinated with sheep virus. The goat virus produced comparable titration results in both sheep and goats, as did the sheep virus.

Table 1. *Cross protection and challenge between sheep and goat pox virus strains*

Species	Virus vaccine used	Challenge virus	Titre
Ovine	F 90 Goat strain	I 306 Sheep	1 } —
			2 } —
			3 } —
			4 } —
	Unvaccinated		5 } 4·8
			6 } 4·5
Caprine	I 306 Sheep strain	F 90 Goat	1 } —
			2 } —
			3 } —
			4 } —
	Unvaccinated		5 } 4·4
			6 } 4·9
Ovine	None	F 90 Goat	4·4
Ovine	None	F 90 Goat	4·6
Caprine	None	I 306 Sheep	4·8
Caprine	None	I 306 Sheep	4·5

*Serological response to vaccination*

A group of 26 sheep were vaccinated with a 1 ml. dose which contained 1000 TCID 50 of virus before inactivation. The antibody response was assayed and expressed as the reciprocal of the log difference between pre- and post-inoculation titres. The results were:

$$1.3 \pm 0.15 \text{ (mean } \pm \text{ s.e.)}$$

All the sera showed fluorescence at 1/10 dilutions when tested by the indirect FAT. There was no generalization of infection amongst any of the animals vaccinated. All developed an induration at the site of inoculation, forming a scab which later sloughed away.

*Contact experiment*

This was carried out to examine the possibility that virulent virus was being excreted in the scab from the inoculation site. Four vaccinated sheep were kept with four susceptible unvaccinated animals. The contact unvaccinated animals all developed lesions of sheep pox within a period of 8 weeks. On post-mortem, lesions were found in the lungs and it is suspected that these were the primary lesions and the route of infection probably by inhalation.

*Field results of the use of the vaccine*

Vaccine was widely used in the face of the outbreaks. In the one instance where importation of sheep from an infected range area resulted in much mixing of immune and non-immune populations and considerable losses from pox virus infections, the use of the vaccine completely prevented further clinical cases. Ring vaccination around infected areas in the southern pastoral area also prevented

further spread. No generalization of infection was reported after the use of the vaccine in the field.

### *Storage*

The vaccine was stored at 4° C. for 1 year and used to vaccinate a group of four sheep. Seroconversion was demonstrated by the indirect fluorescent antibody tests in all four.

### DISCUSSION

It is reasonable to conclude from the epidemiological observations, the characteristics of the viruses, their pathogenicity and immunogenicity that they are identical. The same virus is producing both the sheep and goat disease in Kenya. This absence of any host specificity in the Kenyan sheep and goat pox viruses is notably different from those in the Middle East and India.

A field observation, which was confirmed by serological studies with the indirect fluorescent antibody technique, was the occurrence of silent infections in a large number of animals. Transmission of the virus in the field outbreaks could have been by direct or indirect contact, or inhalation. Contact infection occurred in the laboratory between infected and susceptible sheep in the same pen, and infection of sheep in two adjacent pens followed, presumably by inhalation or by indirect contact via the animal attendant. It was not possible to support the observation by Plowright & Ferris (1958) that contact infection did not readily occur. The pens were fly-proofed.

The indirect fluorescent antibody method was used for diagnosis and epidemiological work, and is probably more useful than the serum neutralization test with this virus. Results obtained by serum neutralization are not a reliable guide to the immune status of the animal, as neutralizing indices are low (Plowright & Ferris, 1958); and misleading (Ramyar & Hessami, 1970). A sequential study of the immunoglobulin responses to the Kenya virus assayed by this method is being carried out. The potency of the current vaccine is assessed by seroconversion by FAT, and results show complete correlation with resistance to challenge.

The fact that wild ruminants were not involved in the outbreaks and have no antibody, would support a view that the virus was introduced at some time into Kenya either from North Africa or India, whence the East African domestic small ruminant population is thought to come.

A successful vaccine was prepared to meet the exigencies of the outbreak. This proved valuable in the control of existing disease outbreaks but it clearly has disadvantages for use where no disease exists. Attempts are being made to attenuate this Kenya virus, and to determine the PD 50 of the current vaccine.

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