THE EPIDEMIOLOGY OF CAMELPOX AND THE DEVELOPMENT OF A CAMELPOX VACCINE

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i

This thesis is my original work and has not been presented for a degree in any other University

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ii

DEDICATION

This work is dedicated to my wife Wangari,and my three boys Gitau,Kariuki and Njogu

TABLE OF CONTENTS

ix

LIST OF TABLES

XV

xvii

xviii

LIST OF APPENDICES

ACKNOWLEDGEMENTS

THE EPIDEMIOLOGY OF CAMELPOX AND THE DEVELOPMENT OF A CAMELPOX VACCINE

ABSTRACT

The role of disease in camel husbandry is little understood although it is known that calf mortality is the most serious problem faced by the people who rear camels. Camelpox is the most serious viral disease affecting camel calves in many parts of the world but its clinical manifestation and prevalence have not been examined in Kenya. Camel *contagious ecthyma*, another closely related and sometimes indistinguishable condition to camelpox has also not been fully investigated. Basic biological characterisation of the camelpox virus has been performed but the propagation and chemical composition of the camelpox virus have not been studied in great detail as with other viruses. There are no reports of efforts at preparing effective vaccines against camelpox.

In this study, the prevalence and clinical manifestation of clinical camelpox as well as camel contagious ecthyma were studied in two main camel rearing districts of Samburu and Turkana in Kenya. Camelpox was found with equal frequency in these districts, but while it was found in two calf herds in Turkana, it was found in two adult herds in Samburu. Camelpox exhibited itself as pox lesions affecting mainly the head where pustules were seen on the muzzle, lips and nostrils. Mandibular and cervical lymph nodes were

swollen. In the adults there was also severe oedema of the neck. Camel contagious ecthyma was found in four herds only in Turkana where it affected camel calves. The lesions were mainly similar to camelpox but was associated with secondary bacterial infection. Goat kids in the herds of affected camels were also severely affected. Camelpox virus was propagated on the chorio-allantoic membrane of embryonated chicken eggs,continuous cell lines(VERO and BHK-21) and on several primary cells(sheep kidney, skin and lung cells,calf kidney and thyroid cells). Sheep kidney cells were found to be the most appropriate in the propagation of camelpox virus where giant cell formation(syncytium) was prominent. Camelpox virus was found to be non-pathogenic to several laboratory animals like rabbits, mice, chicken and rats. Four polypeptides of camelpox virus were identified after polyacrylamide gel electrophoresis. Camelpox virus was inactivated with hydroxylamine hydrochloride, acetylethyleneimine and formalin. Formalin was found to be a more suitable inactivant and was used to inactivate bulk camelpox virus. The prepared virus was then mixed with incomplete Freund's adjuvant and used to vaccinate rabbits which showed an increase in antibody titres against camelpox virus with no side effects. This vaccine was then used to vaccinate twenty camels while ten camels which were not vaccinated were used as controls. All camels were challenged after three weeks by intradermal scarification. The vaccinated camels had small skin lesions which healed within three days. The control camels which had not been vaccinated, had

significantly larger lesions which took about twenty days to heal. The antibody titre was significantly higher in the vaccinated animals than in the control animals.

It was concluded after the survey that camelpox was an important disease in Kenya but whose magnitude was not known before. Camelpox was found to not only affect whole herds, but also affected calves and adults. Camel contagious ecthyma was found in camel calves and mostly associated with caprine outbreaks of contagious ecthyma. Sheep kidney cells were found suitable in preparing bulk vaccine against camelpox. A formalin inactivated camelpox vaccine was found to be effective in protecting calves against the clinical disease. The vaccinates were found to be immune when challenged.

INTRODUCTION

1.

The camel is a particularly useful animal in the dry and arid areas where its ability to withstand high ambient temperatures is a great asset(Hassan, 1971). Other important abilities include staying long without drinking water, eating foliage of poor nutritional value to other animals(Knoess, 1977;Williamson and Payne, 1978), and the padded feet which are suited for desert travel.

The dromedary is numerically far superior than the Bactrian camel and totals almost 90 per cent of the genus Camelus in the world today. According to the F.A.O.(1978), there are about 15 million camels in the world of which 12 million, or more than 80 per cent are found in Africa. In Africa, Somalia has over 5 million camels and Sudan about 3 million camels. Both these countries account for 70 per cent of the camels found in Africa.

Kenya, with 0.6 million together with neighbours Chad(0.4 million) and Ethiopia(1 million) account for 12.5 per cent of the camel population in Africa. The actual population of camels in Kenya (Table 1) was found to be 628,000 camels(Kenya Rangelands and Ecological Monitoring Unit, 1981). More than half(62 per cent)of the camels in Kenya are found in North Eastern Province and Isiolo district(Table I). The camels are essential for the livelihood of nomadic pastoralists who depend on the camels milk for subsistence especially in the dry season.

TABLE I : ESTIMATED NUMBER OF CAMELS IN KENYA'S CAMEL PRODUCING
DISTRICTS 1977-1983

DISTRICT	COUNTING PERIOD	ESTIMATED NUMBER
Wajir	1977,1978	159,485
Mandera	1977,1978	112,225
Isiolo	1977,1978	96,680
Marsabit	1977,1978,1981	89,810
Turkana	1977,1981,1983	75,992
Garissa	1977,1978	45,814
Tana River	1977,1978,1980,1983	33,964
Samburu	1977,1981	11,458
Baringo	1977,1981,1982	1,630
Kitui	1977,1980	1,267
Laikipia	1981,1982	733
TOTAL		628,050

Source:Government of Kenya:Unpublished data from KREMU(Kenya
Rangeland Ecological Monitoring Unit)

The economic potential of the camel is barely tapped, which is
unfortunate, considering that they demonstrate more efficient
utilization of pasture than most other species of livestock
(Fazil,1977). Camel husbandry has, however, been relegated to the

pastoralists. One of the reasons for the unpopularity of the camel, is the long distance covered each day by grazing camels. This has relegated the camels to the outskirts of the arable land, where water is scarce. The second reason is the low reproduction potential of the camel. Females are not fertile until five to six years of age and males not until six to seven years of age (Yagil and Etzion, 1984); that post-partum oestrus occurs only a year after calving(Knoess, 1977), which makes the calving interval to be two years.

The third reason is the high calf mortality which can reach up to 50 per cent in the year old calves(Richard, 1979). Competition between man and calf for the dam's milk is known to play a role but the importance of disease is little understood. Camelpox disease, however, is known to be the most serious viral disease of camels affecting especially the young camels(Wilson et. al., 1980). In a survey of camel sera obtained from camels in different areas of Kenya using serum neutralization test, camelpox was found to be endemic(Davies et.al., 1985). An ELISA test also demonstrated that camelpox is widespread in Kenya and also other neighbouring countries like Sudan and Somalia (Munz et.al.,1986 b).

Although actual cases with clinical signs and epidemiological patterns have not been described and published in Kenya, reports from veterinarians in these areas indicate that the condition is widespread during and after the rainy season.

Camelpox has been reported to be widespread in Somalia (Kriz, 1982), India(Leese, 1909), Iran(Baxby, 1972), Iraq(Falluji

3

et.al., 1979)U.S.S.R.(Krupenko, 1972) and Egypt(Tantawi *et.al.*,

1974). Severely virulent strains of camelpox have been shown to

cause mortalities ranging from 10 to 50 per cent in camel calves

(Kriz, 1982)in Somalia which borders Kenya. Camelpox disease is

particularly significant in pastoral areas, because deaths may also

result from secondary bacterial infections due to the unsanitary

conditions of rearing camels(Mcgrane and Higgins, 1985). Camelpox

is closely related to camel contagious ecthyma another pox

infection in camels, which has been described in Mongolia

(Dashtseren *et.al.*, 1984), Russia (Roslyakov, 1972) and Kenya(Munz

et.al., 1986 a). It has also been reported in Somalia(Kriz, 1982;

Moallin and Zessin, 1988). It is caused by a parapox virus in

contrast to camelpox virus which is an orthopoxvirus. Clinical

surveys have been hampered by the failure to appreciate that pox in

camels (i.e. classical pox and contagious ecthyma) can be caused by

both a parapox virus and an orthopox virus(Roslyakov, 1972;Kriz,

1982). More information is required on the epidemiology of camelpox

and camel contagios ecthyma, so as to reveal the relative

importance of these two virus infections(Baxby, 1982).

Camelpox is well recognized among camel owners. Bedouin camel

herders are said to protect their stock by rubbing a suspension

of pox crusts in milk into the scarified labial surface of the

calves(Mcgrane and Higgins, 1985). Leese(1909) reported a similar

vaccination procedure in the South Eastern part of the Punjab

and in Rajputana.In the U.S.S.R., a camelpox vaccine is reported to

be available(Borisovisch,1974). In the USSR, Sedov(1974) suggested

4

that lesions might be minimized by inoculation of immune serum.There is no other record of a camelpox vaccine.

Camelpox virus belongs to the genus orthopoxvirus but its polypeptides are distinct from polypeptides of other orthopoxviruses(Turner and Baxby, 1979). In further investigations, Turner and Baxby, (1979) found that the core proteins of several species of orthopoxviruses were indistinguishable on a one-dimensional gel but the membrane and subsurface polypeptides showed distinctive differences in polypeptides of the 30K-40K,20K-25K and 50K-60K.

Two groups of orthopoxvirus polypeptides are of particular practical importance:(1)Those involved in immunity and protection and (2)Antigens that are Orthopoxvirus species-specific and therefore useful in viral classification and diagnosis.

In this study therefore, it was planned to study the epidemiological pattern of camelpox and camel contagious ecthyma outbreaks. The clinical manifestation of such outbreaks will be described and any relationships examined. Secondly, the propagation properties of camelpox virus on different tissue cultures and embryonated eggs were planned to be studied with the aim of finding the most convenient propagation method for bulk virus preparation. This is of prime importance in any vaccine development program.

Thirdly,it was the purpose of this study to develop an inactivated vaccine against camelpox, and to test various inactivating agents and that the most efficient one would be used to inactivate camelpox virus. The vaccine combined with an adjuvant

5

was then to be tried on laboratory animals and then tested on camels. Lastly, it was planned to examine the main structural camelpox virus polypeptides and compare to those which have been described by other workers. In future, the immunodominant polypeptides among these polypeptides could be defined and genes for them cloned to be inserted in a suitable vector for the propagation of a genetically engineered vaccine.

REVIEW OF LITERATURE

<u>**TYPES AND DISTRIBUTION OF CAMELS IN EAST AFRICA**</u>

According to Mason and Maule(1960), all camels in East Africa belong to three broad categories often referred to as the Sudanese camel, the Eritrean camel and the Bendir or Somalia camel. This geographical approach to the identification of camels emphasizes the original habitat of the camel. In Kenya, the dominant type is the Somali camel.

Another method of identification utilizes the names of the major tribes which are camel pastoralists. Hence in Kenya, the Somali camel is further distinguished into three types of camels thus; the Somali camel, the Rendille or Gabbra camel and the Turkana camel. The Somali camel is the heaviest, and the best milk, meat and fat producer. However, the Turkana although the smallest in size, has an unusual ability to resist drought while the Gabbra is in between(Sperling, 1985).

It is also possible to identify camels on the basis of their transportation services. This latter approach yields only two types of camels often referred to as the "Baggage camel" and the "riding camel". Fazil(1977) described the "riding camel" as light and finely built with many qualities which make them more agile. On the other hand, the "baggage camels" vary in size and conformation.Some of these types of camels are large and clumsy. Their physique is generally broad, thus making them best suited for carrying loads.

2. 1. 2 REGIONAL DIFFERENCES IN DISTRIBUTION AND DENSITY

Among all livestock, the camel has the most restricted distribution not only in Kenya, but throughout the entire East Africa. According to MacGillivray(1967), the estimated number of camels in Kenya by 1964 was approximately 1,265,000. However, a more recent estimate in 1978 gave the estimate as approximately 640,600(KREMU, 1981). It is important to note that KREMU admitted that some parts of the drylands of Kenya were not surveyed because of poor security. The KREMU field methodology which involved both aerial surveys and sampled ground surveys might not have been the ones employed by MacGillivray(1967) in his estimate of the camel population in East Africa. MacGillivray(1967) found only 1000 in North Eastern Uganda and none in Tanzania.

A more recent survey by KREMU(1981), showed the distribution of camels to be confined mostly to the North of the Equator(Table I). Kenya is therefore, regarded as the southern limit in camel distribution in Africa(Pratt and Gywnne, 1978). In Kenya, camels are kept in the semi-arid and arid areas with a higher density in the arid districts(Table I).

2.1.3 CAMEL PASTORALISM IN KENYA

2.1.3.1 THE CAMEL PEOPLE

The pastoralists in the arid areas of Kenya belong to two major linguistic groups-:

The Cushitic: Somali(Ogaden, Degodia, Ajuran, Aulian and
            Gurreh) Galla(Ormo, Borana, Sekuye),
            Shangilla, Gabbra and Rendille.

The Nilo-Hamitic: Turkana, Samburu(Loikop), Njemps, Tugen,
            Pokot and Maasai.

These are the ethnic groups which are primarily a cattle owning people and who also herd sheep, goats and donkeys. Some of the groups have had camels for a long time while others like the Samburu acquired them recently. The Maasai started acquiring them in 1988.

Commercial ranches have been set up that herd camels as well as other livestock. These are the Ol Maisor ranch and Ngare Ndare in Laikipia district; Galana ranch in the coast province. Although these ranches are mostly in semi-arid areas with relatively more rainfall than the arid areas, camels are said to do well and complement the other animals in providing milk and meat to the pastoralists who look after the livestock (Evans and Powys, 1980).

2.1.4 CAMEL PASTORALISM IN TURKANA DISTRICT

The Turkana people's recent acquisition(last 150 years) of the camel is said to be a "spectacular innovation" which quickly revolutionized their pastoral production system(Odegi-

9

Awuondo, 1985). Its adoption was popular and widespread. They liked the camel basically for four major reasons; Firstly, it was a species of a "cow" which could feed on the vast thorny vegetation which hitherto remained unutilized and thus unprofitable. These could now be put to good use;thanks to the camel.

Secondly, the Turkana discovered, to their immeasurable delight, that this strange animal was extremely resistant to diseases;few diseases attacked it so it remained healthy much of the time. Thirdly, it could withstand the worst drought and was watered only occasionally. It could therefore be deployed in a wide area thus allowing for even population distribution over the vast territories. And fourthly, and perhaps most important for them, was the fact that the camel was their idea of a "hybrid cow", yielding many more times more milk than the ordinary cow;remained in milk much longer and could be milked several times in a day. In addition, camel milk was discovered to posses higher nutritional value than cow's or goat's milk. It is claimed that when they drank it they became strong and vigorous.

The acquisition of the camel, therefore, revolutionized the Turkana economy. From this accidental discovery, the people were able to optimally utilize the land resources, produce more food, feed better, grow healthier, multiply more; and by the mid-19th century had emerged as a major power in the northwestern part of what is today Kenya. They were a conquering power in control of a large area. Their expansionist ambitions were to pit them against the British intruders at the turn of the century, plunging the

10

region into nearly two decades of wars of resistance.

Between 1900-1919 the Turkana lost thousands of their livestock due to the punitive actions of the state(Odegi-Awuondo, 1985). By the early 1920s, the district was already poverty-stricken. Hunger, disease and malnutrition became widespread and have persisted to the present day. Apart from losing large numbers of their stock at the hands of the troops in the inter-war years(1919-1939) and beyond, taxation robbed the district of much of their property. Droughts, livestock diseases and raids from neighbours claimed yet another large portion. The Turkana who did not die from disease and starvation merely survived;their pre-colonial prosperity was no more. Thousands of people became helpless and destitutes. Only the well-to-do, those able to collaborate with the state, remained in possession of fairly large herds. The camel then became a status symbol and even today, the ownership of a camel is a hallmark of prosperity.

2.1.4.1 THE PHYSICAL AND ECO-CLIMATIC SETTING OF TURKANA DISTRICT

Turkana district is rich in natural resources which favour not only camel husbandry but a very viable pastoral economy based on goats and cattle. Turkana-land has an area of 64,048 Sq km, including some sq 2000 km of water(Lake Turkana). It occupies approximately one ninth of Kenya's total land area, making it the second largest district after Marsabit. Its vastness in itself is a major economic asset to the people as it spans over the four eco-

climatic zones III-VI(Odegi- Awuondo, 1985).

Much of the southern, western and northern parts of the district lie in eco-climatic zones III and IV. The land is higher here, rising to about 2000-3000 m above sea level, with their peaks in Loima,Karasuk, Loriu, Pelekech, Lokwanamur and lorianatom Ranges. These areas receive higher rainfall, which in certain parts may be as high as 700-1000 mm.per annum. They also enjoy a higher moisture index of -10 to-30(zone III) and -30 to -40(zone IV). Temperatures are lower, never rising above 30'C even during the hottest days. Large areas have rich volcanic soils suitable for crop agriculture. Much of this part is covered with evergreen forest formation and woody grasslands.

As one descends the escarpment towards the central plains, one enters eco-climatic zone V with an average elevation of 1,200m. It is hotter and drier than the higher grounds. The rainfall seldom exceeds 700m per annum, and the moisture index is from -40 to -50. The temperatures are normally above 30°C. The forests give way to thorn bush comprising of commiphora woodland with various types of *Acacia tortilis*, soft woods, several varieties of palatable browse and succulent grasses. This eco-climatic zone has pockets of sandy rich soils and sandy loams that are suitable for crop-based agriculture.

Zone VI covers the lowest part of the district. These are vast plains stretching to lake Turkana basin. The altitude is between 400-900m., and the rainfall is low, ranging between 200-300 mm per annum with a moisture index of -50 to -60.The temperatures are

extremely high recording mean annual maximum of 36-38°C and mean annual minimum of 22-25° C. The combination of the high rate of evapotranspiration and high temperature experienced in this zone leads to severe aridity. The predominant vegetation is thus annual grasses, dwarf shrubs,scattered stands of *Acacia reficiens subsp micera*, and perennial grasses. The rest of the land is covered with barren sandy and rocky wastes. These extreme climatic conditions in the central plains, where Lodwar the district headquarters stands, influence the outsiders to dismiss the whole region as a district of burning heat and sand waste, harsh and uninviting by any standards(Barber, 1960).The highest numbers of camels are reared in zones VI and V and fewer camels in zone IV (Odegi Awuondo, 1985).

2.1.5 CAMEL PASTORALISM IN SAMBURU

The Samburu are a group of semi-nomadic pastoralists living in North-central Kenya (in "Samburu District"). They are Maasai speakers who have traditionally tended cattle, sheep, goats and donkeys(Sperling, 1985). Untill recently, the Samburu were considered as one of the more successful cattle keepers in East Africa. Within the last twenty five years however, they have experienced a series of crises which have depleted their cattle herds (Sperling, 1985). Widespread raiding between 1964 and 1980; animal diseases (East coast fever and Foot and mouth diseases) have all had their effect, but the major disasters have occurred in the form of drought. In the past twenty five years, the Samburu have

13

suffered through four extensive dry periods; 1959-61, 1965, 1971, 1979-80. In the 1980 drought, the severist so far, Government reports indicated that fifty per cent of the cattle herds were lost in the lowland of Wamba division alone (Sperling, 1985).

2.1.5.1 THE PHYSICAL AND ECO-CLIMATIC SETTING OF SAMBURU DISTRICT

Samburu district is located in North-central Kenya, at the southern end of Lake Turkana. It is an administrative district of 20,809 sq.Km., and approximately 95 % of the Samburu people live there. The district is characteristic of many of the areas used by pastoralists. Lands are arid and semi-arid; and dwarf shrub, bushes and wooded grasslands stand as the prominent vegetation. Soils are generally very poor, most are volcanic, ranging from clays to sandy lcams. Rainfall is erratic, highly localized and unpredictable from year to year.

The divisions of the District (Lorroki,Baragoi and Wamba)are sufficiently distict to be described separately. Lorroki in the south east encompasses the highland plateau, Kirisia grasslands, and an extensive forest reserve. It has one long, reliable rainy season which peaks in July and August(vs. a bimodal rainfall in the other two divisions). It is only here that group ranching and agricultural experiments have been initiated. The division is bounded on the west by a rugged escarpment which effectively isolates it and on the south by the ranching areas of Laikipia.

Baragoi, in contrast, is primarily a division of lowland

plains;its thickly forested Nyiro and Ndoto ranches stand as an oasis in a rocky desert. This division receives the least rainfall. The yearly average ranges between 300 and 350 mm. Most of the District's 14,000 Turkana people live here(sperling, 1985).

Wamba is both the largest division and the host of the largest herds. Cut by the Mathew range, Wamba's Western basin contains the heart of Samburu lowlands;its Eastern plains are now used as dry season reserves. Rendille not Turkana provide the dominant foreign influence in the division.

Lorroki with relatively high rainfall, grass and cooler temperatures, poorly supports camel herding. Baragoi, which is hot and dry, is perfect for camel rearing. Wamba with extensive rangeland has been primarily cattle country but due to land deterioration is now being used more and more for camel rearing(Sperling, 1985).

2.2   THE CAMELPOX DISEASE

2.2.1 DISEASE IN CAMELS

Camelpox is an infectious skin disease of camels caused by a pox virus in the genus Orthopox virus. It is well known among the pastoralists who rear camels in Kenya by different names. The Turkana people refer to it as "Iitune"; the Samburu call it "Ndubi";the Boran/Gabbra call it "Kolmoman"and the Rendille call it

"yahri". Other names used to refer to camelpox outside Kenya include "Djidri"(Tunisia, Algeria, Morocco);"Afrur"(Somalia); "Thaddi"or Chechek(India).

Camelpox is a systemic disease of a cyclic pattern characterized by a pox exanthema over the entire body caused by a poxvirus. It was first described by Leese(1909) in Punjab, India. Several strains of pox viruses isolated from camels have since been reported in Iran(Baxby, 1972), Iraq(Falluji et.al., 1979), U.S.S.R. (Krupenko,1972; Marennikova et.al.,1974), Egypt(Tantawi et.al., 1974), Somalia(Kriz, 1982) and Kenya(Davies et.al., 1975).

The disease is thought to occur in many areas of Africa and Asia where dromedary camels occur but it has not yet been recognized in bactrian camels(Camelus bactrianus). It does not occur among feral dromedary camels in Australia(Fenner et.al., 1989).

The camelpox virus was identified as a virus of a separate identity in the Orthopoxvirus group of the family Poxviridae (Mahnel, 1974; Mahnel and Bartenbach, 1973;Baxby, 1972). Other outbreaks of camelpox disease have been caused by Vaccinia virus (Krupenko, 1972)and a virus resembling Orf(Roslyakov, 1972;Kriz, 1982). It is the most serious and widespread viral disease of camels(Wilson et.al., 1980). Camelpox is a contagious disease which mostly affects young camels two to three years of age and herd outbreaks are often associated with the stress of weaning or poor nutritional status(McGrane and Higgins, 1985).

The disease generally occurs throughout the year and is most

16

serious during the rainy season and it is at this time that deaths are likely to occur. The disease is a generalized exanthema in camels whose features, resemble smallpox in man but it may also occur as a localized infection. The incubation period in natural cases ranges from 10-15 days. The incubation period after experimental exposure is 3-7 days(Pandey *et.al.*, 1985). Benign as well as malignant forms of the disease have been found to occur in affected camels. Foals are more susceptible to infection than adults and the mortality rate is also higher in young stock. In the young camel, the disease appears in a generalized form while in older camels, the disease appears in a localized form(Pandey *et.al.*, 1985)

Within a few days of infection, the disease begins with a mild fever. Papules are usually first seen on the labial mucosa. These progress to vesicles, pustules and eventually scab formation. Diffuse oedema may be present around the lips. During the vesicular stage, the camel rubs its lips to relieve pruritis. As a result the vesicles rupture and may become secondarily infected. Difficulty in eating may be experienced and the camel may loose condition. Local lymph nodes may become enlarged. A brown crust develops over the lesions, which usually heal within three weeks. Mammary glands, genitals, the inguinal and peri-anal region, the thighs and sometimes the feet may also be affected. Corneal opacity may also occur(Pandey *et.al.*, 1985). Affected animals recover in 1-2 months.

The disease spreads horizontally through direct or indirect contact. A more severe form of the disease, reported during the

rains by Leese(1909) caused skin lesions all over the body and a marked fever. In an epidemiologic survey of camelpox, a case fatality rate of 28% was reported by Jazek *et.al.*,(1983). Kriz(1982), reported a virulent form of camelpox in Somalia with lesions concentrated around the eyes and mouth. Lesions were also found on mammary glands, genitalia and anal areas. In 30 camelpox outbreaks in Somalia which were considered, (Kriz, 1982) 295 cases were recorded with 16 deaths among 1052 camels. The mean age of all cases was 2.9 years and that of fatal cases 2.1 years. Sixty eight cases were detected in males and 227 cases in females giving sex-specific prevalence rates of 313 cases per 1000 male camels and 272 cases per 1000 female camels. The mortality rate was higher in males(27.6 deaths per 1000 camels) than in the females(11.9 per 1000). The case fatality rates were 8.8 per cent in males and 4.4 per cent in females. The highest case fatality rate in a single outbreak which occurred in Lower Juba was 28.5 per cent. The highest age-specific fatality rate(13.4 per cent) was in the 0 to 1 year age group.

Different clinically characteristic infections with varying case fatalities were also recorded by Kriz(1982). For example in a pilot study, the case fatality rates in five camelpox outbreaks in Badade district of Somalia, ranged from 10 to 50 per cent. In another district of Kismayo, out of 20 camels which had camelpox, none died while 10 progeny born of these camels suffered from camelpox and seven died. Both of these outbreaks occurred in the months of April to June and there was no apparent difference in

18

prevailing living conditions or nutritional status. It therefore seems that camelpox disease, can be manifested in a mild form or severe form. Camels recovering from the disease may develop life-long immunity (Pandey et.al., 1985). Young foals born of recovered mothers are usually resistant to infection during the early part of life. Mortality rates in young camels vary from 4 to 7% (Pandey et.al., 1985)

Diagnosis of the disease is based on the history, course of disease and clinical signs(Pandey et.al., 1985). Confirmation of infection depends on isolation and characterization of the aetiologic agent and is based on structure, pock morphology, ceiling temperature, lack of growth on rabbit skin, pathogenicity for adult mice, cytopathogenic effect in Hela cells, giant cell formation and mortality of 3-day old chickens(Pandey et.al.,1985).

Death is a sequel to generalized infection and secondary bacterial invasion. If the animal dies after a severe septicaemia secondary to bacterial infection, the carcass is emaciated and fetid(Pandey et.al., 1985). Pocks in various stages may be found on the whole body. In non-fatal cases with secondary bacterial infection, localized abscesses can be observed even in visceral tissues, especially those of the throat. If the conjunctivae are involved, permanent unilateral or bilateral blindness may result (Jazek et.al., 1983).

The clinical camelpox disease has not been described in Kenya except for the characterisation of camelpox virus (Davies *et.al.*, 1975).

2.2.2 POTENTIAL RISK OF CAMELPOX FOR HUMAN INFECTION

The key to smallpox disease eradication in man, was aggressive surveillance-containment activity and eradication was confirmed by extension of these surveillance techniques."Smallpox and its eradication" provides detail on all certification exercises(Fenner et.al.,1988). The National certification exercises were reviewed by independent international commissions and in 1980 the WHO General assembly accepted the proposal that smallpox had been eradicated (WHO,1980).

Due to the presentation of camelpox as a severe generalized exanthema resembling smallpox in man rather than the more localized infection which characterizes bovine cowpox, camelpox has received some interest from workers as a possible human infection. Camelpox is caused by an orthopoxvirus and the virus when studied by conventional techniques has certain resemblances to smallpox virus(Baxby, 1972;1974). Furthermore, camelpox occurs in areas where smallpox had been endemic for a long time(Kriz, 1982).

Kriz(1982) investigated thirty nomadic groups in two regions of south-western Somalia in respect of camelpox outbreaks(Table II). Out of two hundred and eighty six persons, ninety five people or 33 per cent had been vaccinated against Smallpox. The latter

20

were asked if they had seen or heard of skin rashes on people handling camelpox cases and they were also physically examined for presence of any rash. Out of these ninety five people, only one person, a member of a nomadic group settled in Sakow district of Middle Juba region had heard of such a case. This incident was investigated and it was found that a 40 year old male who had contact with infected animals and was not vaccinated against smallpox had developed a rash after 3 days of general discomfort.

There were only 4 lesions on his arm, all appeared at one time and passed through vesicular,pustular and scab stages. Scabs fell off after 14 to 15 days of rash leaving white-pink scars 7 to 8 mm diameter which resembled smallpox scars. This patient was not seen by a World health organisation smallpox eradication program(SEP) until 5 days after the scabs had fallen and so no specimens were taken(Kriz,1982). Serum from this patient was tested and gave a positive haemagglutination inhibition test for orthopoxvirus antibodies. Specimens taken from sick animals belonging to the patient's group were found to be EM positive for orthopox virus, and camelpox virus was isolated (Table II). The area was then thoroughly re-investigated and only two other human rash cases, were found.

(Table II): FINDINGS AMONG CAMEL HERDSMEN WITH CAMELPOX INFECTIONS
AFTER VACCINATION WITH SMALLPOX VACCINE IN SOMALIA

No. OF NOMADIC GROUPS	No.OF PERSONS	No. PERSONS:VACCINATION & SCAR PRESENT (PER CENT)	NO. OF RASH CASES DETECTED (PER CENT)
30	286	95 (33.2)	3 (1.05)

After further searches, only the individual already mentioned
out of the 1916 members of 144 nomadic groups contacted by
epidemiologists in this study was found with a suspected camelpox
infection. The conclusion was that camelpox, although economically
important was of little significance to man in respect of
production of variola-like symptoms in the area investigated.
Nevertheless, the author suggested the need for a wider
immunological survey for camelpox virus antibodies to determine its
pathogenicity for man.

Jazek et.al., (1983) in a survey of 465 camel herdsmen handling
affected camels, reported that these herdsmen rarely developed skin
eruptions and skin specimen taken from them were negative for
poxvirus. From an estimated 20,000 persons at risk, only one report
of a possible case of camelpox was observed and that, too, could
not be confirmed on laboratory examination. The authors concluded

that camelpox is mainly of economic importance due to relatively high mortality, loss in condition and fall in milk production and weight of affected camels. It is not likely that the disease is communicable to man.

2.3   CAMEL   CONTAGIOUS   ECTHYMA

Camel contagious ecthyma (CCE) is also called "Auzdyk" and in Kenya is locally referred to as "Afuturo" by the Samburu and the Rendille.It is caused by parapox viruses which have been isolated in the Soviet Union(Roslyakov,1972); Mongolia(Khokhoo,1982; Dashtseren et.al.,1984) and in Somalia(Jezek et.al.,1983).Usually the CCE is characterised by localized pox-like lesions around the oral   cavity   ,on   the   muzzle   and   nostril(Jazek,1983;Munz et.al.,1986[a]) but generalized lesions have also been described.

Camel contagious ecthyma(CCE) is a disease of camels caused by a   parapox   virus   but   whose   clinical   manifestation   is indistinguishable from camelpox. The CCE virus has been reported by Kriz(1982), in outbreaks of camelpox (orthopoxvirus) in the south west of Somalia. Virus isolates from specimens taken from diseased camels were identified at the centre for disease control, Atlanta and two types of poxviruses identified. An orthopox virus corresponding to the camelpox strains isolated in Iran(Baxby, 1972) and Kenya(Davies *et.al.*, 1975), and a parapox virus similar to the strain of virus which Roslyakov(1972), refers to as causing " Auzdyk"(contagious dermatitis of camels).

23

A similar outbreak of "Auzdyk" involving two year old calves was reported recently in Somalia(Moalin and Zessin, 1988). Pustules and scabs on the lips and nostrils with one case of swellings on the entire head were observed in the affected animals. Electron microscopy revealed typical ovoid parapox virus particles confirming the presence of CCE in central Somalia, where camelpox virus infection was known to have occurred(Moallin and Zessin,1988).

There are no reports of successful propagation of the CCE virus,except in Mongolia where it was propagated on the chorio-allantoic membrane of embryonated chicken eggs(Dashtseren et.al., 1984). One worker has however indicated that CCE was successfully propagated on tissue culture causing cell lysis in about 24 hours(Black, D.[1990], pers.comm.). It was as if there was cell toxicity and yet virus was re-isolated.

In the outbreak in Kenya reported by Munz et.al., (1986 a), camels in Laikipia especially two years or younger, developed proliferative lesions on the lips with occasional extension to the mucosa of the mouth and nostrils. Lesions were reported to occur generally involving the distal parts of the legs, inner parts of the thighs and in females, in the vaginal area. In the initial stages, congested papules appeared. The lips, nostrils and sometimes the entire head became swollen as described for camelpox virus infection(Jazek et.al., 1983). The papules progressed to pustules before encrusting. Finally, the scabs became dark brown and dropped off after six to ten weeks. In the severely affected

24

animals, round black and hairless scars with slightly thickened epidermis remained even after six months. In the less severely affected animals, the scabs dropped off and tissues healed without scarring. Scabs obtained from most of these animals revealed typical ovoid parapox virus particles(Munz *et. al.*, 1986 a). Though animals of both sexes and of all ages were affected, the severity of the dermatosis was much greater in the calves and immature camels. The morbidity in these two age groups amounted to almost 100 per cent, as compared to 10-20 per cent in adult camels. The mortality rate in all age groups was zero.

Due to the swollen mouthparts, browsing was difficult and the camels lost weight and became emaciated. In addition, due to the mechanical irritation by *Acacia* thorns, the pronounced pustules commonly developed secondary infection. In cases of extensive lesions on the feet, infection led to lameness. In 20 out of 21 samples of affected skin scabs, typical parapox virus samples were seen. Their morphology resembled ovine *Ecthyma contagiosum* virus. The ovoid particles measured 230-285 nm X 157-171 nm(average 250 X 160 nm)in size. Their axis ratio was 1:1.56 and their surface showed a regular crisscross pattern(Munz *et.al.*, 1986 a).

What is also significant is that the outbreak occurred during a period of very severe drought and cases of *Ecthyma Contagiosum* in sheep were observed during the same period on the same farm. The details of the outbreak in sheep were however, not given(Munz *et.al.*,1986[a]).

Camel contagious ecthyma closely resembles contagious pustular dermatitis in sheep and goats clinically. In addition, Clinical outbreaks of contagious pustular dermatitis in sheep have been reported in farms where there were outbreaks of camel contagious ecthyma have been reported(Munz et al., 1986 [a])

Contagious pustular dermatitis is a pox virus disease of sheep and goats that is transmissible to man. The first description of the disease in sheep was made by Walley(1890) but since that time, it has been reported in sheep raising areas throughout the world. The disease in sheep and goats is normally referred to as contagious pustular dermatitis(CPD) and orf refers to the disease in man(Robinson and Balassu, 1981).

In naturally occurring typical cases of the disease, it is assumed that the virus enters the host through abrasions on the skin of the lips and face(Robinson and Balassu, 1981). Such abrasions can be caused by thistles, hard stubbles, prickles of red burr, or similar plants(Boughton and Hardy, 1935;Gardiner et.al., 1967). The virus can also be transmitted to the udder of ewes by clinically affected sucking lambs(Robinson and Balassu, 1981).

The virus is highly epitheliotropic, and the lesions produced are usually seen on the nostrils and commissures of the lips. In some cases it may affect the skin, mucosa or other parts of the body, for example, the thigh, axilla, coronet, vulva or udder(Aynaud, 1923;Glover, 1928;Howarth, 1929;Valder et.al.,1979).

26

In the more commonly seen mild conditions, the lesions in the sheep and goats start as discrete reddened swellings on the lips, followed by papules, vesicles, pustules and ulcer formation in three to four days. In uncomplicated cases, the disease is afebrile and self-limiting. Scabs form within one week and peel off the skin within three to four weeks, usually leaving no scar(Aynaud, 1923; Glover, 1928;Howarth, 1929;Schmidt and Hardy, 1932).

Some serious cases besides involving the lips and nostrils, spread to other parts of the body for example, the thigh, axilla, coronet, vulva or udder(Aynaud, 1923;Glover, 1928;Howarth, 1929; Valder et.al., 1979). Another serious case was reported among sheep in Kent, by Darbyshire(1961). The infection persisted in the acute form for two months and involved the gastro-intestinal tract, lungs and heart. A mortality rate of 18 % was recorded. Another serious outbreak was reported in Germany, where lambs and sheep were involved with a high mortality in lambs. In addition to foot and genital lesions, oesophagitis was observed. Many of the affected lambs developed lameness and lost their hooves although no secondary infection was mentioned(Valder et.al., 1979).

The disease is more common during spring and summer mainly among lambs and kids(Aynaud,1923;Glover,1928;Schmidt and Hardy, 1932). The prevalence is lower in older animals, probably because of immunity from either vaccination or past infection(Bruner and Gillespie, 1973). Morbidity can be very high, approaching 100 %(Schmidt and hardy, 1932;Gardiner et.al., 1967), but the mortality rate in uncomplicated cases rarely exceeds 1% (Glover, 1928;Schmidt

and Hardy, 1932). With secondary complications mortality rates may range from 20 to 50 % (Aynaud, 1923;Jacotot, 1924). Production losses from CPD in lambs are due to the lesions on the lips and mouth, which interfere with feeding, or ewes with infected teats refusing to feed their young, subsequently affecting growth rates (Howarth, 1929;Bruner and Gillespie, 1973). Deaths are usually due to complications caused by, for instance, the invasion of the lesion by the screw worm fly *Cochliomyia americana* (Boughton and Hardy, 1935) or *Fusobacterium necrophorum* (Newsom and Cross 1931, 1934; Marsh and Tunnicliffe 1937). A case of CPD associated with *Dermatophilus congolensis* has been reported in New zealand (Cooper et.al., 1970);these authors also cited a report of secondary complications due to streptothricosis among sheep in Kenya(Munz, 1969).

Epidemiological studies revealed early on that the CPD virus is very hardy and is found in high titre in scabs formed during the course of the disease (Aynaud, 1923). It was therefore believed that the virus persists in the environment in dust and wool and this was the source of new infections(Theiler, 1928;Glover, 1932-33;Boughton and Hardy, 1935). Typically the disease is seen in susceptible animals which are usually lambs on property that does not vaccinate. The scabs formed on these animals and the virus they contain adds to the pool of virus in the environment (Robinson and Balassu, 1981).

Once an animal in a flock becomes infected, it is also likely that virus from the lesions that develop will contribute to

28

infections in the same outbreak. This traditional view of the epidemiology has been questioned by Romero-Mercado., et.al., (1973 a,b) who had difficulty finding virus in scabs found late in the course of the disease. They felt that this conflicted with the idea that virus was shed on the ground in scabs acting as a source of infection. In an apparent extrapolation from these findings, it is stated by Robertson(1976) that the source of outbreaks is latently infected carrier animals, although there is insufficient evidence at the moment to support this view(Robinson and Balassu, 1981).

Although CPD is regarded as a disease primarily of domestic sheep and goats, it has also been reported in the musk ox (Kummenege and Krogsrud, 1978), reindeer(Kummenege and Krogsrud, 1979), mountain goats and big horn sheep(Connel, 1954; Samuel et.al., 1975), chamois and thar(Daniel and Christie, 1963), and steenbok and alpacas(Robertson, 1976). These infections were thought to be of ovine or caprine origin. There is also an unusual outbreak reported in dogs (Wilkinson et.al., 1970). It was suggested that the dogs were infected after being fed with unskinned sheep carcases. Most of the outbreaks in these animals resemble the condition in caprine and ovine animals involving mostly the head(Robinson and Balassu, 1981).

Human orf also normally follows contact with infected sheep and is thus an occupational disease of farmers, sheep shears, freezing workers and veterinarians(Purdy, 1955). It is, however, seen in people not directly involved with handling sheep or goats(Kewish, 1951). These infections probably arise from fomites, such as sheep

yard rails, wool hooks and clothing. Man to man transmission has been reported(Lang, 1961). The presence of wounds and abrasions on the arms and hands increases risk of infection(Pask *et.al*,. 1951). Lesions usually occur singly,but multiple lesions do occur. The progression of the lesion is the same as in sheep (Robinson and Balassu, 1981).

2.5   THE CAMELPOX VIRUS

The camelpox virus belongs to the poxviridae family and was identified as a separate species in the genus orthopoxvirus(Mahnel and Bartenbach, 1973;Mahnel, 1974;Baxby et.al., 1975). In terms of size, shape and structure, physico-chemical properties and replication, it resembles vaccinia virus. Furthermore, vaccinia virus causes lesions similar to those caused by camelpox in camels (Krupenko, 1972). Lesions similar to those of camelpox are also caused by a parapox virus(Roslyakov, 1972).

Camelpox viruses isolated from different countries have been referred to as "strains". The Etha 78 strain of camelpox was isolated in Iraq(Falluji *et.al*., 1979). The Tehran strain was isolated in Iran(Ramyar and Hessami,1972). The Egyptian strain of camelpox was isolated by Tantawi and others (1974), while the camelpox strain H520 was isolated in Kenya by Davies and others (1975).

PROPAGATION OF CAMELPOX VIRUS

2.5.1.1   EMBRYONATED EGGS:

The pock lesions formed by the majority of camelpox virus strains on the chorio-allantoic membrane of the developing chicken embryo are small, opaque and white without any central areas of necrosis.

The Kenyan strain of camelpox virus, H520(Davies *et.al.*, 1975) produced pock lesions of a proliferative nature when propagated on the chorio-allantoic membrane of chicken embryonated eggs. Incubation at 37^0 C gave pock lesions which were grey-white and 0.5 to 1mm. in diameter. At 34^0 C, the pock lesions were 0.2-0.4 mm. in diameter and were slightly domed. There was no central haemorrhage or necrosis. No pock lesions were formed at 39^0C. A vaccinia virus used as a control, produced pock lesions at all three incubation temperatures with differing pock morphology at all three temperatures. Histopathological examination of the affected chorio-allantoic membrane showed eosinophilic, intra-cytoplasmic inclusion bodies.

The Etha strain of camelpox from Iraq(Falluji *et.al.*, 1979) produced lesions which were localized at the inoculation area, when propagated on the chorio-allantoic membrane of embryonated eggs. The lesions which occurred on the 5th day were white, 0.4-0.6mm. in diameter, without haemorrhage or necrosis and were not elevated above the surface of the membrane. The virus grew readily at 34, 37

and 39(+ or-0.5)^{0}C. It was not lethal to chick embryos even in high doses(10^5-10^6 Plaque forming units).

The Egyptian strain of camelpox virus (Tantawi *et.al.*, 1974), produced a primary lesion 2-5mm. in diameter when propagated on 11 day old embryonated eggs. The lesion was raised above the surface of the membrane and was surrounded by an inflammatory oedematous area in which were scattered secondary lesions. The secondary lesions were of two types,some were minute, pinheaded, greyish white in colour, while others were large, 1-2mm. in diameter and not raised above the surface of the membrane. Histopathological examination of the infected chorio-allantoic membrane showed that the pock lesions consisted mainly of hyperplasia of ectodermal and mesodermal cells, marked infiltration with eosinophils and lymphocytes. Some cells were destroyed, ballooned and vacuolated. In ectodermal cells, cytoplasmic eosinophilic inclusion bodies were detected.

The Tehran strain(Ramyar and·Hessami, 1972) of camelpox virus was propagated on 9 day old embryonated· eggs and produced distinct pock lesions on the chorio-allantoic membranes, similar to those caused by vaccinia virus.

Some camelpox strains also produce haemorrhagic pocks(Sadykov, 1970;Tantawi *et.al.*, 1978). Marennikova and co-workers (1974) are of the opinion that camelpox virus produces two types of pocks depending upon the temperature of incubation of the chick embryo following inoculation.

The pock lesions are monomorphic, white and pinpoint in size (0.2-0.3 mm) at 37^{0}C, whereas at 34.5^{0}C the pocks are flatter with a haemorrhagic centre.

2.5.1.2   TISSUE CULTURE

The H520 strain of camelpox virus(Davies *et.al.*, 1975) produced cytopathic effects in baby hamster kidney cells, Vero cells, lamb testis, lamb kidney and calf kidney cells. The effects were observed as foci of infected cells which eventually peeled off. Coverslips stained with haematoxylin and eosin showed intracytoplasmic inclusion bodies. The inclusions stained green with Acridine Orange and red with the Feulgen reaction similar to the manner control Deoxy-ribonucleic acid stained.

The H520 strain of camelpox virus produced plaques in Baby hamster kidney cells in petri dishes,in VERO cell cultures, lamb testis and calf kidney cells. The plaques formed, remained small, from 0.4 to 0.8mm. in diameter after five days and were irregularly round in outline.

The Etha strain of camelpox virus(Falluji *et.al.*, 1979) was propagated on chicken embryo fibroblasts and produced a cytopathic effect characterised by cell rounding and detachment from the glass. Small syncytia and cell ballooning were observed. The virus produced plaques under an agar overlay which had lysed centres,sharp edges and an average diameter of 1.2mm.

The Tehran strain of camelpox virus(Ramyar and Hessami, 1972)

33

was propagated on lamb kidney,lamb testis, calf kidney, calf testis, camel kidney, pig kidney, Baby Hamster kidney and vero cells producing clear cytopathic effects.

The Egyptian strain of camelpox virus(Tantawi *et.al*.,1974)was found to replicate slowly in camel embryo and calf kidney primary cultures. Four days post infection, a marked cell loss and many giant cells appeared in the cultures. Eight days post infection, the majority of cells were destroyed and detached from glass. When stained, the preparations showed giant cells or syncytia which usually contained about 20 to 25 nuclei. Cytoplasmic inclusion bodies were evident.

Baxby (1974), was able to propagate camelpox virus on Hela cells,transformed Green monkey kidney cells and transformed human amnion cells. With high doses (10' - 10' pfu/ml), cytopathogenic effect(CPE) was detected within 24 hr and soon spread to involve the whole cell sheet.With lower doses(10' - 10' pfu/ml) CPE was not detected until the second or third day and resulted in localized plaque production. Multinucleate giant ·cells were produced in all these transformed cells.

Other secondary tissue cultures on which camelpox virus has been propagated include Hela cells (Bedson, 1972) and Porcine renal IB-RS 2 cells in liquid medium(Nguyen *et.al*.,1989).The ability to form multinucleate giant cells by camelpox virus in Hela, BS-C-1(Transformed Green monkey kidney) and human amnion cells has been used to differentiate it from variola which only produces rounding up of cells in these cell lines (Bedson, 1972;Baxby, 1974).

34

2.5.1.3 INCLUSION BODIES

Depending on the species of orthopoxvirus, one or two types of inclusion bodies may be found in infected cells. Irregular, weakly staining inclusion bodies were first described in cells infected with variola and vaccinia viruses by Guarnieri(1892) and used to be called "Guarnieri bodies." Much more prominent, eosinophilic inclusion bodies are found in cells infected with ectromelia virus (Marchal, 1930), cowpox virus(Downie, 1930) and raccoon poxvirus(Patel et.al., 1986). In a systematic study, Kato et.al., (1959) proposed that the Guarnieri-type inclusion bodies be called "B-type" and the Marchal-Downie inclusion bodies "A-type". B-type inclusion bodies are found in all poxvirus-infected cells, and are the sites of poxvirus replication(Fenner et.al., 1989). The type of inclusion bodies formed by camelpox virus has not been described.

2.5.2 PHYSICAL AND BIOCHEMICAL CHARACTERISTICS OF CAMELPOX VIRUS

2.5.2.1 MORPHOLOGY:

Electron micrographs of the Tehran strain of camelpox virus(Ramyar and Hessami, 1972) showed that it is closely related to other pox viruses and has a diameter of approximately 270 um. Camelpox virus is brick shaped and is 280 X 180 nm in size(Marennikova et.al., 1974;Roslyakov, 1972).

35

2.5.2.2 HAEMAGGLUTINATING ACTIVITY

The Etha strain of camelpox virus(Falluji *et.al*.,1979) from Iraq was found to agglutinate chicken red blood cells at pH 6, 7 and 8 at room temperature but with a low Haemagglutinating titre(1/8) in comparison with that of vaccinia virus(1/256). It did not agglutinate red blood cells from different species of mammals (Horse, ox, sheep, goat, human type O, rabbit and dog). The haemagglutination could be completely and partially inhibited by anti-camelpox and anti-Vaccinia sera, respectively.

Similarly, the H520 strain of camelpox from Kenya(Davies *et.al*., 1975) was found to be poorly haemagglutinating chicken red blood cells(1/8)as compared to Vaccinia virus(1/64).

The Egyptian strain of camelpox virus(Tantawi *et.al*., 1974) was, however, found not to have any agglutination effect on mammalian or chicken red blood cells.

2.5.2.3 HAEMADSORPTION;

Haemadsorption activity has been demonstrated with the H520 strain of camelpox virus(Davies *et.al*., 1975)using chicken red blood cells.

The H520 strain of camelpox(Davies *et.al.*, 1975) was found to be sensitive to chloroform but not to ether. Chloroform reduced the titre from $10^{4.6}$ TCID to $10^{1.8}$TCID$_{50}$/0.1ml.

The Egyptian strain of camelpox virus (Tantawi *et.al.*, 1974), was found to be resistant to ether but sensitive to chloroform. The Etha strain of camelpox virus(Falluji *et.al.*, 1979) was resistant to the action of ether and chloroform. The Tehran strain of camelpox virus(Ramyar and Hessami, 1972) was found to be ether and chloroform sensitive, exhibiting a fall in titre of more than four logs.

2.5.2.5 THERMOSTABILITY:

The Tehran strain of camelpox virus(Ramyar and Hessami, 1972) showed a reduction in titre of more than 2 or 4 logs when heated for 5 and 10 minutes, respectively at 37 ^{0} C. The virus heated at 56^{0}C for 15 and 20 minutes appeared to be completely inactivated. The Etha strain of camelpox virus(Falluji *et.al.*, 1979), however, was resistant to heat at 56^{0}C for one hour. A reduction of 50 per cent of virus infectivity and complete inactivation occurred at 60^{0}C after 1 and 2 hours, respectively. The virus infectivity was greatly reduced at 70^{0}C after 10 minutes and completely disappeared after 30 minutes.

2.5.2.6 pH RESISTANCE

The H520 strain of camelpox virus(Davies et.al., 1975) was reported to be sensitive to pH in the range 3-5 and again between pH 8.5 and pH 10. The Etha strain of camelpox virus(Falluji *et.al.*, 1979)remained infective and was unaffected at a pH range between 3 and 8.5 for 1 hour.

2.5.2.7 CAMELPOX POLYPEPTIDES

Camelpox virus, like vaccinia virus has a complex structure in which four components can be distinguished(Westwood *et.al.*, 1964):(1)the biconcave core(Peters, 1960;Easterbrook, 1966); (2)the lateral bodies;(3)the outer protein coat;and (4)the outer envelope (Westwood et.al., 1964;Medzon and Bauer, 1970). The polypeptides which make up vaccinia virions were analyzed by Sodium Dodecyl Sulfate(SDS) polyacrylamide gel electrophoresis and seventeen structural polypeptides three of which were associated with the core were identified (Holowczak and Joklik, 1967).

Katz and Moss(1970) showed that some of these polypeptide peaks could be further separated into two or three polypeptides, thus increasing the total number of resolved structural polypeptides to 22. The vaccinia virus genome is large enough to code for between 200 and 400 average sized proteins, the coding capacity expressed in total molecular weight of protein being between 8 X 10^6 and 16 X 10^6. About 80 virus coded polypeptides,

38

with a total molecular weight of 3 X 10^6 were demonstrated by Pennington (1974). He however, noted that it is likely that some polypeptides are synthesized in amounts too small to be detected by the methods used and in addition, some polypeptides probably escaped detection due to their masking by residual host protein synthesis early in infection (Pennington, 1974). Some different virus polypeptides with similar or identical molecular weight also co-migrate and cannot therefore be resolved.

The most recent study of the number of virus specific-polypeptides synthesized in vaccinia virus-infected cells(Carrasco and Bravo, 1986), utilized two dimensional electrophoresis and revealed a total of 279 polypeptides. Since the total estimated molecular weights of these proteins exceeds the coding capacity of the vaccinia virus genome, some are undoubtedly products of the processing of the others. On the other hand, the figure is an underestimate in that some polypeptides may not have entered the gels and small polypeptides(<10K)would not have been detected.

Camelpox has been differentiated from vaccinia by the absence of p 53 and from all other orthopox viruses by the possession of p31 in addition to p32 and p 35 instead of p23 present in all the orthopox viruses(Turner and Baxby, 1979).

2.5.2.8 ANTIGENIC PROPERTY

Camelpox virus is distinguishable from variola virus in the laboratory and is considered to be a separate species of the

orthopox virus genus(Baxby, 1974;Marennikova *et.al.*, 1974). Tests made at the Communicable Disease Centre, Atlanta, USA, on the material received from Somalia have identified two types of pox viruses in camels:an orthopox virus corresponding to camelpox strain isolated in Iran, USSR and Kenya and parapox similar to a strain which Roslyakov (1972), refers to as "Auzdyk"(contagious dermatitis of camels) strain (Pandey *et.al.*, 1985).

According to Jazek *et.al.*,(1983) at least four different strains of camelpox have been reported from various parts of the world:(1). An Iranian strain that closely resembles variola virus(Baxby, 1972), strain T-72 which resembles monkeypox virus in some biological properties (Marennikova *et.al.*, 1974), (3). The Ramyar and Hessami strain which resembles vaccinia virus in pock morphology on the chorio-allantoic membrane; Ramyar and Hessami(1972), and (4). The Roslyakov strain(Roslyakov, 1972).

2.5.3   PATHOGENICITY

Generally, camelpox virus has a narrow host range. The H520 strain of camelpox virus(Davies *et.al.*, 1975) did not produce any reactions in rabbits or sheep after intradermal inoculation or scarification. Vaccinia, on the other hand, produced hyperaemia, oedema and necrosis at the inoculation site. No reaction of camelpox virus was observed in defoliated feather follicles of day old chicks. The H520 showed a very low pathogenicity for chick embryos which were alive after five days incubation. In infant

40

mice, the LD50 for H520 was $10^{3.4}$ TCID $_{50}$/.02 ml compared with a figure of $>10^6$ for Vaccinia.

The Egyptian strain of camelpox virus(Tantawi *et.al.*, 1974) was found to be pathogenic to camels. Intradermal inoculations, produced nodular small lesions at the site of inoculation ten days post infection. Rabbits which had been inoculated intradermally reacted with a localized nodular and ulcerative skin lesion. Mice were susceptible to intracerebral inoculation, showing nervous manifestation and death within one week. Guinea pigs were refractory to experimental infection.

The Etha strain of camelpox virus (Falluji *et.al.*, 1979) produced in rabbit skin, a mild transient erythema which disappeared on the 5th day after inoculation and produced in monkeys, typical localized pox lesions from which the virus could be re-isolated. The multiplication of the virus in the monkeys was also evident by serological tests. The virus produced no reactions in chickens, mice and guinea pigs. In contrast, vaccinia virus produced hyperaemia and nodular lesions in the rabbit inoculated by scarification and in 3 day old chicks, typical vesicular pox lesions appeared.

The Tehran strain of camelpox virus(Ramyar and Hessami, 1972) was reported to cause generalized pock lesions and death occurred when young camels, 9 to 10 months old were inoculated. Cattle, sheep, goats and small laboratory animals were refractory to infection.

Baxby *et.al.*, (1975), was interested in the behaviour of

41

camelpox virus and smallpox virus in camels, since the two have similarities in standard tests used in the diagnosis of smallpox. Camelpox virus inoculated by intradermal route in two camels was infective at the lowest dose tested(1.8×10^{-1}TCID$_{50}$/ml.). Papules started to develop after 5 days and progressed through pustules and vesicles by 6 to 8 days. Crusts began to form by 9 to 10 days.

Secondary generalized lesions appeared on day 9 and 11, with the same sequence of development as primary lesions. Recovery in the two animals had occurred after 4 weeks. Virus was isolated from the primary and secondary lesions and on day 7, from the blood of both animals. Smallpox virus on the other hand was unable to infect camels. This reinforces the differences of the two viruses in tissue culture and in gel diffusion tests. Infection was initiated at 1.8 by 10^{-1} TCID$_{50}$/ml as titrated in lamb kidney cells. This indicated the lower sensitivity of camelpox virus as compared to intradermal inoculation in the camel.

Baxby(1972), using a camelpox virus strain(CM-G2) isolated from Iran(Gorgan, Caspian sea area) obtained localized lesions in two rhesus monkeys.

2.6 THE ROLE OF VACCINATION IN THE CONTROL OF POX DISEASES

Vaccination against pox diseases has had a long history. Variolation, the inoculation of smallpox material into a susceptible person in order to provide immunity, has been used for hundreds of years and in all parts of the world. The first

description of the procedure was given about 500 A.D. in China and India(Kahn, 1963). The Chinese powdered old crusts and applied them to the nostrils;Brahmins in India preserved crusts and inoculated them into the skin of the unscarred;Persians ingested crusts from patients;It has been used in Africa since earliest times, in Europe since the 1670's, and in the Americas. Mather indicated in 1716 that the procedure was used by slaves who had learned the technique in Africa(Leikand,1942).

Lady Mary Montague's account in 1716 of variolation in Turkey describes the gathering of 15 or 16 people to be inoculated at one time(Dixon, 1962). It was this practice that Lady Mary Wortley Montague, wife of the British Ambassador to Turkey, introduced into England in 1718. Crusts and vesicle fluids were selected from patients during epidemics of mild disease reffered to as alastrim). The practice spread to the colonies, where it was more widely used than in the British Isles, but it never became popular because of the risks involved.

In England, variolation was practised on a limited scale in the early 18th century. The morbidity and mortality associated with the procedure apparently prevented mass applications until the Sutton family in the 1760's, began to inoculate with a minimal amount of virus and very light scratches. Mortality decreased and mass inoculation programs became possible. The Sutton family claimed to have inoculated about 55,000 people by 1768, and 300,000 by 1776(Razzel, 1951).

Variolation was so widespread in England, that its results

43

may have been responsible for the acceleration in population growth which occurred in the middle of the 18th century(Razzel, 1951). The established practice of variolation in England protected British troops, while smallpox decimated the American army during the crucial battle for Quebec in June 1776(Bernstein, 1951). Opposition to variolation in America, prevented President George Washington from obtaining official sanction from congress to variolate the military until January 1777. This sanction resulted in one of the most extensive mass immunization campaigns to that date.

The exact origin of vaccination (as contrasted to variolation)is still the subject of historic debate. Fewster, an English physician, presented a paper on cowpox and its ability to prevent smallpox to the London Medical Society in 1765. In 1769, Bose, in Germany noted a similar relationship. In 1774, Benjamin Jesty is said to have protected his wife and three children with cowpox(Kahn, 1963). Most historians credit Jenner with the introduction of vaccination. On May 14, 1796, Dr.Edward Jenner inoculated material from a cowpox lesion into the arm of James Phillip. One week later, the boy developed axillary pain and a slight fever. On July 1, material from a smallpox pustule was inoculated in both arms of the boy but no disease followed. With this, a new era of medicine was introduced and the possibility of controlling diseases through preventive inoculations became a reality. Jenner thus added scientific testing to the previous observations that cowpox could protect against smallpox. He also

44

recorded his observations and made them publicly available. His first attempt to have the observations published by the Royal society was rejected as the Society felt there was lack of adequate proof(Kahn,1963). In 1798, Jenner himself published the findings under the title "An inquiry into the causes and effects of the variolae vaccine"(Jenner, 1798), a disease discovered in some of the Western counties of England particularly Gloucestershire,and known by the name of cowpox".

By 1799, vaccine had been sent to more than 100 physicians all over Europe and by 1800, vaccine had been introduced to the United States(Kahn,1963). In 1800, vaccine was also introduced to India and Ceylon, Spain, and generally around the world. Mass vaccination programs were instituted and by 1803, about 17,000 people had been vaccinated in Germany. In 1805, Napoleon ordered the vaccination of all his troops. In a 10 year period (1804 to 1814), two million people were vaccinated in Russia and by 1816, compulsory vaccination had been instituted in Bavaria, Denmark, Sweden, France and certain parts of Italy(Dixon, 1962).

The vaccination program was not as successful in Africa, South America and the middle east especially due to loss in potency of the vaccine. This improved after development of freeze-drying and a full scale global eradication of Smallpox was started in 1967. The last known Smallpox case was detected in 1977 in Merka town, Southern Somalia(WHO,1978). The success of the worldwide WHO Smallpox eradication programme is living testimony of the success of vaccination.

Though vaccination with variola is relatively safe, it gives rise to rare but occasionally fatal complications affecting the skin or central nervous system, especially with initial vaccinations. One type results from widespread secondary implantation of virus on skin diseases, such as eczema(eczema vaccinatum) or even diaper rash. Viraemia frequently occurs in vaccinees who have agammaglobulinemia or dysgammaglobulinemia, and results often in fatal disease(Generalized vaccinia). A more frequent and less serious form of generalized vaccinia appears to be related to an allergic reaction, since viraemia is apparently rare, virus is usually absent from the skin lesions, and vesicles do not form erythematous urticarial reactions (Fenner *et.al.*, 1989).

Probably the most alarming complication is progressive spread of a primary vaccination response with extensive necrosis of skin and muscle(Vaccinia gangrenosa), in those rare persons with thymic dysplasia,who cannot develop cellular immunity. Postvaccinal encephalitis another serious and often fatal form of demyelinating disease with an incidence of about 2.9 per million primary vaccinations can also occur. These unpleasant and occasionally serious side effects of Jennerian vaccination have over the years stimulated many studies on the antigenicity of inactivated vaccinia virus, with the objective of using inactive preparations as substitutes for live virus. Most investigations have been directed to the problem of immunogenicity of inactivated vaccinia virus.

The problem with inactivated vaccinia preparations is that there are antigenic differences between the surface antigens of

46

enveloped and non enveloped virions of vaccinia virus both of which are infectious. The infection provoked by vaccination with live virus(with both enveloped and non-enveloped virions) led to the development of a full range of humoral and cellular immune responses. In contrast, inactivated vaccine failed to provoke a humoral response to the envelope antigens(Appleyard *et.al.*, 1971:Turner and Squires, 1971:Payne, 1980).

The use of inactivated vaccinia tended to produce less protection than that from live virus. Rabbits immunised with large doses of vaccinia virus inactivated by formaldehyde or ultraviolet irradiation developed extremely high titers of neutralizing antibody(tested against nonenveloped virions), but they remained susceptible to generalized rabbitpox infection, although they were protected from death(Boulter *et.al.*, 1971). Antisera against inactivated virus with very high levels of neutralizing antibody to nonenveloped, but none to enveloped virions, provided a weaker protection against challenge infection than apparently much lower titer of antibody induced by infectious virus, which, however contained both kinds of neutralizing antibody(Boulter *et.al.*, 1971).

Nevertheless, in an effort to reduce the incidence and severity of postvaccinal encephalitis, formalin inactivated vaccine("vaccinia-antigen") was used in Germany during the late 1960s for a "priming"vaccination, followed by vaccination with standard vaccine. Subsequently, Marennikova and Macevic(1975) showed that pre-immunization of rabbits with vaccine inactivated by

47

^{60}Co gamma-irradiation greatly enhanced their response to vaccination with active vaccine given 7-60 days later, both in the titer of antibody produced and in its rate of production. Pre-immunization reduced the incidence of viraemia in the rabbits 4-5 days after vaccination with live virus. Preliminary human trials on the use of this preparation as a priming antigen were carried out in eastern Europe in 1977, but by this time global eradication of smallpox was imminent, and no further trials were made(Fenner et.al., 1989).

Many of the pox virus diseases are controlled by the use of live attenuated vaccines although inactivated vaccines have been used especially in the control of sheeppox and smallpox.

Early attempts to inactivate sheeppox virus with formaldehyde (Duclox and Cordier, 1926) butanol, ethyl ether(Mori, 1922) and crystal violet(Angeloff, 1940) were not successful. Celiker and Arik(1968) stated that a merthiolate inactivated gel adsorbed sheeppox vaccine conferred immunity for 12 months. A vaccine prepared with a Beta-propiolactone inactivated virus mixed with sodium alginate or oil adjuvant has been reported to provide dependable immunity(Uppal, 1963;Sharma and Dhanda, 1970).

An adsorbed sheeppox vaccine inactivated with merthiolate (Rafyi and Mirchamsy, 1956) was used in Iran. More than 24 million doses were used in 8 years producing a dependable immunity of 12 months in the control of sheeppox. This vaccine was found to be better than live vaccine as the latter produced serious reactions and abortions. The live vaccine also had the potential to be spread

48

to non infected animals.

Hoffman(1951), Deply *et.al.*, (1953), Celiker and Arik(1962) and Dias vigario(1965), have reported favourable results with formalized gel adsorbed vaccines. Another successful inactivated sheeppox vaccine was developed by Davies(1976). Sheeppox virus propagated on Baby Hamster Kidney cells or lamb testis was harvested by freeze thawing and used if the titre was $10^{5.5}$ TCID/$_{50}$ or greater. It was diluted with phosphate buffered saline to give approximately 1000 $TCID_{50}$/ml of final suspension, and mixed with an equal volume of alhydrogel at 4^0C. The virus, saline and alhydrogel mixture was stirred for 48 hours at 4^0C. Merthiolate was then added to a final dilution of 1/10,000 and also 0.03%formalin. The vaccine administered intramuscularly was found to control field outbreaks of sheeppox especially in the range areas of Kenya(Davies, 1976).

The failure to develop a potent inactivated vaccine has been said to be due to lack of understanding of the kinetics of virus inactivation by the various inactivating agents(Goyal and Singh, 1975). The controlled conditions for inactivation of sheeppox virus were determined by Goyal and Singh(1975), such that sufficient inactivation was achieved without loss of immunogenicity. Three chemicals viz;Formaldehyde, hydroxylamine and N-acetylethyleneimine were used to make vaccines from a single batch of high-titred sheeppox virus adapted in lamb testicular cells.

There only mention of a camelpox virus vaccine is in Russia where a vaccine is reported to be in use but no details are provided (Sedov, 1974). Bedouin camel herders are also reported to

49

control camelpox by rubbing pox crusts from infected cases in milk and applying these on the scarified labial surface of young calves(Mcgrane and Higgins, 1985). There is no other report on the use or development of a camelpox vaccine.

2.6.1 THE CHOICE OF AN IMMUNIZATION METHOD AGAINST ORTHOPOX VIRUS INFECTIONS

Apart from smallpox,protection against orthopoxvirus infections is justified in only a few situations.The most important are with laboratory animals namely,-mousepox in laboratory mice and rabbitpox in laboratory rabbits. In Germany, cowpox virus infection of zoo and circus elephants is deemed sufficiently dangerous to these valuable animals to justify vaccination, and if economic considerations permitted, immunization of camels against camelpox would be justified(Fenner *et.al.*,1989)

Here in Kenya, the search for a camelpox vaccine is justified in many ways. Camels in Kenya are reared in the arid and semi-arid areas of Kenya which constitute about two-thirds of the total land surface. With such a huge area, it is surprising that the total population of camels is only 628,000. It is even more significant when one considers that these animals are primarily reared by pastoralists whose only means of survival is in rearing the animals.During drought, more than 60 % of these herds are decimated putting these pastoralists at the brink of extinction due to starvation.

The camel with its superior adaptation to survive and even thrive in drought conditions, would appear to be a good solution during those dry times. Although the low reproductive rate of the camel is partly to blame for the low camel population, the rôle of disease although poorly understood is obviously of tremendous importance. Camelpox,the most serious viral disease of camels is therefore one area where medical intervention would seem most appropriate in improving camel herd production. The question still remains, however, as to which would be the most appropriate vaccine to use. At the outset, a live vaccinia vaccine would appear to be a solution but this is accompanied by the danger of spreading to susceptible camels and causing a generalized skin disease and possibly in man(Fenner et.al.,1989)."Variolation",or the use of live camelpox material from actual cases of camelpox to vaccinate young camels before the onset of rains is still used by some camel herders like bedouins to vaccinate camels.However,this practice is accompanied by the risk of the disease proliferating to full scale camelpox especially if the camels are immunocompromised or malnourished.

Another alternative would be to use a passaged camelpox virus strain. The problem with this strategy is that complete attenuation would require many passages in the order of 500 as for example the attenuated strain of vaccinia obtained after 572 passages in chick embryo fibroblasts(Hochstein-Mintzel et.al.,1975).

Currently, the Samburu and Turkana pastoralists manage camel contagious ecthyma and camelpox cases by branding. The affected

lymph nodes, mostly the cervical and mandibular, are branded with a red hot iron. The actual benefit from this treatment is doubtful except that the lymph nodes will regress in size due to atrophy after being physically destroyed by heat. The theory behind it is to arrest the progress of the virus before spreading to other areas. By the time swelling is observed on the lymph nodes however, the virus has already replicated there.

A compromise solution would appear to be an inactivated camelpox vaccine since it would be easy to produce and have no potential dangers of reverting to virulence. It would also be easy to transport and it would not require refrigeration, a prerequisite for live vaccines. This is significant in arid and semi-arid areas as ambient temperatures can at times get to 30^0 C or over.

2.7   INACTIVATION OF POX VIRUSES

Of the pox viruses, the most commonly inactivated pox virus has been the Vaccinia virus(Ramano Rao, 1962; Turner *et. al.*, 1970; McNeil, 1965), followed by sheeppox virus(Goyal and Singh, 1975). There is no report on the inactivation of camelpox virus.

FORMALDEHYDE INACTIVATION

Formaldehyde is widely used for the production of inactivated vaccines. It reacts mainly with amino groups in proteins. It replaces labile H atoms on $-NH_2$ and -OH groups which are abundant in proteins and nucleic acids, and also on -COOH and -SH groups of proteins. Virions with double stranded nucleic acid like pox viruses are inactivated mostly by modification of the protein. In the presence of formaldehyde, the helical structure of the nucleic acid melts at relatively low temperatures, freeing the amino groups. (Davis, 1980). The inactivation curve is of the multicomponent type and therefore extrapolation always involves a risk as the dose may be underestimated and a dangerously higher level of virus could remain(Davis, 1980).

This was demonstrated by Salk when poliovirus was inactivated in one week by 1:4000 formalin, pH 7 at 37^0C(Davis, 1980). When purified virus was used, the inactivation followed pseudo-first order kinetics but when crude virus preparations were used the exponential rate of inactivation was not constant and the curve tailed off markedly. This led to many problems exemplified by an incident in which residual infectious virus in several lots of commercial vaccine induced 260 cases of poliomyelitis with 10 deaths. These errors were recognised and rectified by the use of 1M $Mgcl_2$, when inactivation by formalin shows much less tailing off. Extensive controlled studies showed an effective protection against paralytic poliomyelitis in 70-90% of those immunized.

53

Formalin has been used widely to inactivate many viruses. It was used to inactivate vaccinia virus by mixing an equal volume of purified virus and 0.12 M formalin, 0.04 M glycine (McNeil, 1965). The virus was completely inactivated after 48 hr at 19^{0}C. The virus was removed from inactivant by differential centrifugation.

Formalin inactivated vaccinia virus was found to be more antigenic than acetylethyleneimine inactivated vaccinia virus(Ramano Rao,1962). This is the converse to the findings by Brown and Crick(1959) with foot and mouth virus.The foot and mouth virus was inactivated in 4 hours at 37^0 C while vaccinia was still infective after 6 hours and 24 hours was allowed for complete inactivation,(Ramano Rao, 1962). The longer period for inactivation was suggested to be the cause of reduced antigenicity and a shorter period of inactivation recommended.

In the inactivation of vaccinia by Ramano Rao(1962), 0.012M formaldehyde was used to completely inactivate vaccinia by 48 hr at 37^{0}C. The virus was removed from the inactivant by differential centrifugation.

Formalin was used by Turner et.al., (1970) at 19^0 C for 24 hr in the presence of formaldehyde(0.03M) and glycine(0.04M) to inactivate vaccinia virus.

In the Goyal and Singh(1975) inactivation trials, sheeppox virus was placed in bottles at 37^{0}C, and formalin added such that a final concentration of 1:2000(0.006 M formaldehyde and 1:4000(0.003M) formaldehyde respectively were obtained. Bottles were withdrawn every 8 hours starting from 0 hr and excess

54

formaldehyde neutralized with an equimolar concentration of sodium bisulphite solution(Singh, 1960). The samples were then dialysed against two changes of Hanks buffered saline at 4^0C for 48 hr. and titrated for residual infectivity. Formalin was shown to have a first order reaction kinetics in its inactivation of sheeppox. The time required for inactivation at 37^0C, as determined by extrapolation was 40 and 56 hr by 1:2,000 and 1:4,000 formalin, respectively(Goyal and Singh, 1975)

2.7.2   HYDROXYLAMINE INACTIVATION

Hydroxylamine is thought to react exclusively with nucleic acid(Lie, 1964). It acts as a mutagenic agent by converting cytosine to a derivative that pairs with adenine, producing a guanidine, cytosine-adenine, thymine transition which is not reversed by hydroxylamine(Davis, 1980).

Hydroxylamine hydrochloride has been used to inactivate viruses and to prepare experimental inactivated vaccines against fowl pest, Influenza and foot and mouth disease viruses(Schafer and Rott, 1962;Fellowes, 1966). Viruses differ considerably in their susceptibility to this reagent(Franklin and Wecker, 1959).

Of the pox viruses, vaccinia has been inactivated with hydroxylamine. In one of such trials, hydroxylamine hydrochloride was freshly prepared as a 2M solution and the free acid neutralized with sodium hydroxide(Turner et.al., 1970), before final dilution to a final concentration of 0.1M in the virus mixture. Inactivation

was found to be complete after 60-72 hr at 18-20⁰C in the dark(Turner *et.al.*, 1970).

In a similar trial, McNeil(1965) used a 0.002 M preparation of hydroxylamine hydrochloride to inactivate equal volumes of vaccinia virus. There was complete inactivation from 1.6×10^3 pfu/ml to nil, after 16 hours at 19⁰C.

In the inactivation of sheeppox virus by Goyal and Singh(1975), hydroxylamine hydrochloride($NH_2OH.HCL$) was freshly prepared as 4 and 2M solutions. The free acid was neutralized with NAOH (Fellowes, 1966), and this solution was added to the virus suspension at 37⁰ C to achieve final concentrations of 1, 0.5, 0.2, 0.1 and 0.05 M hydroxylamine. The volume of virus-inactivator mixture was kept constant at 2 ml. After suitable time intervals, the samples were removed and the action of hydroxylamine was stopped by the addition of 8 ml cold phosphate buffered saline(1:5) to each sample. After dialysing twice against phosphate buffered saline, the samples were titrated for residual infectivity. The inactivation was of the first order kinetics and the time required for inactivation upto the base line with 1.0; 0.5, 0.2, 0.1 and 0.05 M hydroxylamine solutions was 16, 24, 32, 40 and 48 hr, respectively.

2.7.3   ACETYLETHYLENEIMINE(AEI) INACTIVATION

Acetylethyleneimine was found to be markedly effective in inactivating foot-and-mouth disease virus and the vaccine obtained

56

was found effective in immunising guinea pigs against foot-and-mouth disease virus(Brown and Crick, 1959). This is still the most commonly used inactivating agent for foot-and-mouth disease virus. When acetylethyleneimine was compared to formaldehyde in the inactivation of vaccinia virus(Ramano Rao, 1962), 24hr were needed for complete inactivation with AEI while 48hr were needed with formaldehyde although both were used at 1:1000. AEI was found to harm the antigenicity as judged by production of neutralizing antibody, to a greater extent than inactivation with formaldehyde. This is the converse of the findings by Brown and Crick, (1959) with Foot-and-mouth disease virus who were able to inactivate foot-and-mouth disease virus in 4hr at 37 ^{0}C. The longer period used for the inactivation of vaccinia was thought to contribute to the loss in potency of the vaccine in immunising rabbits.

In the inactivation of sheeppox virus by Goyal and Singh(1975), Sheeppox virus in 2ml volumes at 37 ^{0}C was placed in MaCartney bottles and 2 ml of 0.1% AEI($H_2C-N-CH_3$) added to achieve a final concentration of 0.05%AEI(v/v). The samples were removed every 2 hr and excess AEI was neutralized by adding 0.4ml of 20% sodium thiosulphate(Brown and Crick,1959). After dialysis against phosphate buffered saline for 24hr, residual virus infectivity was titrated. Acetylethyleneimine was found to inactivate sheeppox virus by first order kinetics reaction. A time of 12hr was needed by extrapolation for complete inactivation of sheeppox virus by 0.05% AEI.

57

IMMUNIZATION TRIALS AND CHALLENGE

INACTIVATED VACCINES AND TRIALS

Several immunisation and challenge trials of live and inactivated pox vaccines have been done. The most commonly used virus in these trials has been vaccinia virus propagated on embryonated eggs. In one of these trials, vaccinia virus inactivated with acetylethyleneimine or hydroxylamine was compared to live virus in its immunogenic effects in immunising rabbits(Ramano Rao, 1962). Five animals were used in each group and a second vaccination was done after 4 weeks. The immunised animals were challenged 17 weeks after immunisation by intradermal inoculation of serial 10-fold dilutions of live virus. The reactions were observed daily for 12 days. Sera were obtained before and after immunisation and neutralizing antibodies tested for(Ramano Rao, 1962). The results suggested that AEI vaccine elicited a weaker antibody response than formaldehyde vaccine.

In a similar trial(Turner et.al., 1970), the immunogenicity of vaccinia virus inactivated by different agents(dye, ultraviolet light, heat, formalin, hydroxylamine, B-propiolactone) was tested. Antibody response and skin immunity were tested in rabbits. Each vaccine was inoculated intramuscularly in four rabbits in two 1 ml. doses given two weeks apart. Four rabbits were given live virus and four uninoculated rabbits served as control. One week after challenge, 0.1 ml. of serial fivefold dilutions of live virus was

applied to the skin of scarified rabbits and skin reaction observed, just as in potency assays of smallpox vaccine. The challenge virus doses causing confluent or semi-confluent lesions on the skin of control animals produced only scanty or single lesions in animals immunised with most of the inactivated vaccines, indicating that they conferred a considerable degree of skin immunity.

Lesions in immunised animals developed like those in unimmunized animals upto the fifth day, but thereafter their evolution was accelerated. The individual pocks remained circumscribed, with moderate erythema surrounding them. There was no secondary spread, their mildly necrotic centre developed eschars by the seventh day, and healing preceded that of control animals by several days. No lesions were produced in rabbits immunized with live virus. The profile of neutralising, complement fixation and haemagglutination inhibition antibodies was followed before and after immunisation. There was little correlation between serum antibody concentration measured by the three methods; they were unrelated to the skin immunity induced in the same rabbits and they did not indicate the superiority of a particular inactivator.

Another trial of a sheeppox inactivated vaccine was preformed by Goyal and Singh, (1975). Three vaccines were prepared by inactivation of sheeppox virus with either 1:4000 formalin(for 56 hr), with 0.05M hydroxylamine(48 hr), or 0.05% acetylethyleneimine (12 hr). The vaccines are each inoculated into ten different sheep with 6 sheep kept as controls. Half the number of animals were

59

immunized subcutaneously with 0.5ml of vaccine and the rest were inoculated at 4 spots intradermally with 0.2ml inoculum at each site. On day 21 post-inoculation, all the animals including unvaccinated controls were challenged intradermally with serial tenfold dilutions of virulent virus containing from $10^{3.5}$ to $10^{7.5}$ $TCID_{50}/0.2$ ml. Each virus dilution was inoculated at 4 spots in a row, using 0.2ml virus at each spot. The challenged sheep were observed for 20 days and their thermal and local reactions recorded. There was no local or generalized infection in animals vaccinated with hydroxylamine or AEI. There was no rise in temperature and the animals resisted challenge, whereas the unvaccinated sheep died showing local and generalized infection with a high temperature. Two of the 5 sheep inoculated subcutaneously with formaldehyde-inactivated sheeppox virus showed a little local reaction with highest virus dose ($10^{7.5}$ $TCID_{50}/ml.$), whereas those inoculated intradermally did not exhibit any untoward reaction.

2.8.2   DETECTION OF THE LEVEL OF PROTECTION

The detection of the level of protection by pox vaccines was described early when describing the skin responses of individuals after vaccination with vaccinia virus in the control of smallpox(Elisberg *et.al.*, 1956). The vaccine is administered intradermally by gently breaking the skin under a drop of vaccine. Puncture or scarification permits infectious virus to enter the

skin where it multiplies in the deeper layers of the epidermis. The extent of multiplication and spread of virus, and thus the type of reaction that ensues, depend on the immunity (and hypersensitivity) of the host.

The primary response occurs in an individual who has no effective immunity. A small papule appears on day 3, by day 6 it becomes a vesicle and by day 9 it is a pustule beginning crust formation. By day 11 a crust is formed and this crust is shed by day 17 leaving an area of desquamation and discolouration. A scar is visible upto day 42(Elisberg et.al., 1956).

An accelerated (vaccinoid)response progresses through the same stages as in the primary reaction but does so more rapidly and with less intensity because the subject has partial immunity.

The immediate response is seen on day 1 as a slight swelling which enlarges upto day 5 and then clears away. The early or immediate response is primarily a delayed hypersensitivity reaction. It does not require viral multiplication since it is due to the host reaction to viral protein, and in contrast to the primary and accelerated reactions, the early response can be elicited by a killed noninfectious virus(inactivated by heat at 56^0C for 30 min.). Failure to elicit any dermal response is sometimes seen, but it is never the result of complete immunity;it simply indicates that the vaccination technique was faulty or the vaccine inadequate.

Many vaccine trials have used this skin response as an indicator of immunity. In testing the potency of attenuated sheep

pox vaccines, Martin *et.al.*,(1973) used a dermal titration on vaccinated and control sheep. At least four sheep were used to test the immunizing power of each vaccine. After a 3 weeks period post vaccination, sheep were challenged with virulent virus by injecting 0.2 ml volumes of virus intradermally at each of 4 sites per 10-fold dilution. Five dilutions were injected on one side of each vaccinated sheep. At least one control sheep was included in each test. The level of protection to challenge was assessed on the size, duration and titre of the skin reaction compared with the control sheep and on the presence or absence of a fever greater than 40.5^0C.

Immunity was thus graded into 4 categories:

Complete: There was no dermal response or a transient hypersensitivity-type reaction was observed but this disappeared by the 4th or 5th day after which no thickening of the skin or subcutis could be detected.

Good: The reactions were small after the dermal titre of the challenge virus was reduced by at least 2 $\log_{10}$ units.

Partial:The reactions were less than half the diameter of those of the controls after the dermal titre was reduced by between 1 and 2 $\log_{10}$ unit.

None: Fever and skin reactions of similar size, duration and type similar to those of the control sheep, were observed. There was swelling upto a maximum of about 4 cm followed by necrosis of the overlying skin.

These changes were basically similar to those described by plowright et.al.,(1959).

Another closely related trial is that described by Pye(1990) in assessing protection of sheep vaccinated with a live cell culture grown orf virus vaccine. Vaccinated and non-vaccinated sheep were challenged 5 weeks post-vaccination. Two or three animals were used for each vaccine. Protected animals had small lesions(<3mm). By day 15, the crusty scabs had peeled to reveal healed tissue(secondary response). The animals which were not protected had large lesions(>5 mm). The lesions were swollen and pus filled and healing took 30 to 35 days(Primary response).

2.8.3   NEUTRALIZATION TESTS

Various serum neutralization tests have been performed to assay the antibody responses in animals experimentally infected with poxviruses. Sera from sheep experimentally infected with sheeppox virus have been analyzed by the constant virus varying serum method which appears to be the most commonly used method(Davies and Otema, 1981; Kitching and Taylor, 1985).

The test is performed in culture tubes or microtitre plates. In the latter, which is more commonly used, sera inactivated at 56 ^{0}C is placed in 0.05ml volumes and diluted serially in maintenance medium in doubling dilutions. Virus at 100 TCID $_{50}$/ml is then placed in 0.05ml volumes in the wells and the two are incubated at 37^{0}C for 2 hr. Primary tissue culture cells are then added at 1 X 10^6

63

cells/ml in 0, 1ml volumes and the plates incubated. Uninfected cultures are maintained with all tests. The serum titre is that dilution suppressing 50 % viral cytopathic effect.

2.8.4   CAMELPOX:

There are no reports of immunization and challenge trials involving camelpox virus in the literature

MATERIALS AND METHODS

3.1 EPIDEMIOLOGICAL SURVEILLANCE

3.1.1 TURKANA DISTRICT

For the epidemiological investigation of camelpox, Turkana district was chosen. There were several reasons why Turkana district was chosen as an area to investigate the epidemiology of camelpox. The main reason was that it was accessible in that it was possible to traverse this district from one end to the other. This was made possible by the camel project based in Turkana and funded by the Norad Project. There was no limit on the travel and a four-wheel drive vehicle was freely available.

The other main reason was that the district is a typical area where camel rearing is practised by pastoralists and it is arid with minimal areas available for arable farming. It is therefore an area which can be compared to other camel rearing districts like Marsabit, Mandera, Wajir, and other arid districts although the population of camels is not very high at 89,810 (Table I).

Turkana district was therefore visited and extensive enquiries made in all the main administrative locations. Camels were physically examined, and from the ones with suspicious lesions, skin scabs were obtained. A total of 600 camels in 30 herds ranging from 2-40 camels were observed and examined. Enquiries were made on the past or present signs of camelpox observed by pastoralists.

sera was obtained from 30 animals in two herds with suspicious camelpox symptoms. Camel sera from apparently healthy camels was obtained from 100 camels. From four herds with suspected contagious ecthyma outbreaks, skin scabs and sera were also obtained. The total number of camels in these four herds was 200. Skin scabs were also obtained from goat kids which were similarly affected in the same herds. The total number of goats in these herds was 250.

3.1.2   SAMBURU DISTRICT

Although not as extensively studied as Turkana district, fifteen camel herds in Samburu district were also investigated. Camel farmers in scattered homesteads were visited. Five hundred camels in fifteen herds ranging from 6-70 camels were examined. Similar enquiries about the occurrence of camelpox were made. In one of these herds, a group of 60 camels brought from Galana to Kisima in Samburu and which were reported to have an outbreak of camelpox were examined. Skin scabs from twenty camels and 35 sera samples from these animals were obtained. Another herd of 45 adult camels which were moved from Somalia to Wamba, Samburu and which had an outbreak of camelpox were physically examined and 30 scabs and serum samples obtained. Sera was also obtained from 30 apparently healthy camels from two herds in the same area. In Laikipia Ngare Ndare ranch with 30 camels on the ranch was visited and camels examined.

Skin scabs obtained from suspected cases of poxvirus infection were ground and re-suspended in a minimum volume of phosphate buffered saline. A Formvar electron microscope grid was floated on a drop of virus suspension for two minutes, removed and blotted with the edge of a blotting paper, and placed on a drop of 2 % sodium phosphotungstate pH 6.6. After 90 s the grid was blotted, air dried and examined in a Zeiss EM 10 C/R transmission electron microscope operating at 60,000 V.

3.3   TISSUE CULTURE PREPARATION

3.3.1   PREPARATION:

Primary tissue cultures were prepared from bovine kidney, bovine thyroid, bovine lung, bovine skin, sheep kidney. Sheep lung, sheep testis, sheep skin, and sheep testis. The respective organs were cut into small cubes aseptically, using a pair of scissors. They were washed several times in phosphate buffered saline to remove red blood cells and debris. The minced tissues were transferred to a sterile conical flask containing a sterile silicone covered magnet. A volume of 20 ml of a 0.25% trypsin solution was added and the suspension was trypsinised on a magnetic stirrer unit at 37°C for 20 minutes. The cloudy supernate was discarded. The harvest was kept on ice to prevent the action of trypsin. This trypsinisation

67

procedure was repeated and harvests pooled. The packed cells were re-suspended in 10 ml amounts of maintenance medium without foetal calf serum. The cells were pipetted severally to desegregate them. A volume of 0.1ml cell suspension was mixed with 0.9ml trypan blue and the cells counted in a modified haemocytometer. The cell concentration was adjusted to a concentration of 1×10^6 cells/ml with growth medium and dispensed in medical flat bottles. The cells were incubated at 37^0 C until they had formed a complete monolayer after which the growth medium was replaced with maintenance medium.

3.3.2   PASSAGING

Passaging was performed for both continuous cell lines and primary cell lines. The continuous cell lines used were the Baby Hamster Kidney cells and the Vero cells. Cells on confluent monolayers to be passaged were rinsed in phosphate buffered saline(pH 7.2). Two mls of trypsin-versene mixture(1:3) were then added and the bottle incubated at 37^0 C until the cell sheet was freed from the glass. The cell suspension was re-suspended in maintenance medium and then centrifuged at 1000 g for 10 min. The supernate was discarded and the cells re-suspended in growth medium before a cell count was performed as before. The cells were then seeded in the appropriate growth vessel and then incubated at 37^0 C.

3.3.3 FREEZING AND THAWING OF CELLS FOR PRESERVATION

Suspension of cell cultures were preserved as described by Hay(1986). Basically, confluent monolayer cells were trypsinized and collected in a pellet form after centrifugation at 1000 x g for 10 min. A cell count was performed and cells adjusted to a concentration of 10^5-10^7 cells/ml. The cells were re-suspended in a freeze medium at room temperature. The freeze medium was made of growth medium, but with 15 % calf serum and 10 % dimethylsulfoxide. The cells were then dispensed in 5 ml amounts in bijou bottles and placed at 4^0C for 6 hours and then placed at -80^0C.

When cells were to be used after freezing, the Bijou bottles were rapidly thawed in a waterbath(37^0 C). The contents were transferred to a 20 ml universal bottle and 10 ml of growth medium added. The bottle was centrifuged for 10 min at 1000 x g. The supernate was removed and fresh aliquots of growth medium added. The suspension was then cultured in culture vessels as before.

3.3.4 VIRUS ISOLATION FROM SKIN SCABS

Skin scabs from suspected cases of poxvirus infections were ground in a mortar and pestle and freeze thawed three times. A minimal amount of phosphate buffered saline was then added. The suspension was then clarified by centrifugation at 3,000 x g for 15 min. The supernate was then filtered using 0.22 u filters. This preparation was then inoculated in sheep kidney cells and 10 day

69

old embryonated eggs. These were then observed daily for any changes.

3.3.5 INOCULATION OF CAMELPOX VIRUS

The maintenance medium for culture bottles with complete monolayers was discarded and the bottles rinsed twice with sterile phosphate buffered saline. One ml of the virus suspension was placed in each bottle and adsorbed at 37^0C for one hour. The virus was diluted such that the approximate virus:cell ratio was 1:20 or a multiplicity of infection(m.o.i) of 1:20). The virus suspension was then discarded, the bottle rinsed twice with phosphate buffered saline and the maintenance medium replaced. The bottles were incubated at 37^0C and cell death examined daily with an inverted microscope.

3.3.6 HARVESTING OF CAMELPOX VIRUS FROM CELL CULTURES

Camelpox virus was harvested after observing 60% cell death. The bottles were freeze thawed three times. The cell suspension was centrifuged at 1000 x g for 30 minutes and the supernate was assayed for titre and then kept as stock virus at -70^0C.

TITRATION OF CAMELPOX VIRUS

All virus preparations were assayed for infectivity before storage. The virus suspension was first diluted in maintenance medium in round bottomed microtitre plates. This was accomplished by adding 0.2 ml of virus suspension to 1.80 ml of maintenance medium. This tenfold log dilution was repeated from 10^{-1} to 10^{-9} using a

separate pipette each time.

Healthy monolayers of sheep kidney cells were used for titration. Cells were trypsinised as before and 0.1 ml of cells suspension seeded in each of 96 wells in microtitre plates(Flow labs). A volume of 50 ul of the diluted virus suspension was then placed in each well in quadruplicates in the seeded cells. This was repeated for all the dilutions. The plates were then gently rocked and then incubated at $37^{0}C$ in a carbon dioxide incubator(5%). Cell damage was checked daily. The virus titre ($TCID_{50}/ml$) was then calculated by the method of Spearman and Karber as recommended by Finney(1952).

PLAQUE TYPE DEMONSTRATION

Camelpox virus was inoculated onto confluent monolayers in 6 by 4 well- tissue culture plates. After complete virus infection with consequent syncytia formation, the tissue culture fluid was sucked by negative pressure and then a 1% preparation of Crystal violet in

1% formalin placed in 2 ml volumes in the wells. After complete staining overnight, the Crystal violet was sucked off and the stained plaques observed under an inverted microscope.

3.3.9 NEUTRALIZATION TESTS

Neutralization tests were performed in 96 well microtitre plates(Flow laboratories). Maintenance medium in 50 ul volumes was placed in all the wells. Field sera was inactivated at 56^0C for 30 minute and 50 ul volumes placed in quadruplicates in the first wells. Serial doubling dilutions were then performed with a multichannel pipette and the last 50 ul volumes in the pipettes discarded.Cell culture camelpox virus at 100 $TCID_{50}$/ml in 50 ul volumes was then placed in all the wells. The plates were covered and incubated at 37^0C for 1 hr and then overnight at 4^0C. The plates were then kept in a carbon dioxide incubator(5% Co_2) at 37^0C and cytopathic effects examined daily. The serum titre was the titre showing 50 % cytopathic effect. Altogether, 130 serum samples from camels in Turkana and 105 serum samples from Samburu were tested. The sera of thirty experimental camels obtained on various days as described later, were also tested.

3.3.10 PROPAGATION OF CAMELPOX VIRUS IN EMBRYONATED EGGS

Embryonated eggs were inoculated on the chorioallantoic membrane with 100 ul camelpox virus obtained from Samburu using an

aseptic standard technique as described by McCarthy and Dumbell (1961). All eggs were removed 9 days after inoculation. The eggs were first chilled and then aseptically swabbed before the shell was broken. The chorio-allantoic membrane was broken and the inner contents tipped into a petri dish. The chorio-allantoic membrane which was left adhering to the eggshell membrane was then removed with a pair of forceps and rinsed in sterile saline. It was then examined for pock lesions.

3.3.11　　　　　PREPARATION OF BULK CAMELPOX VIRUS

Bulk preparations of camelpox virus from embryonated eggs were prepared by pooling harvests from one hundred infected embryonated eggs. Chorio-allantoic membranes which had many pock lesions some of which were fused were pooled together in 20 ml universal bottles and preserved at -20 ^{0}C. These were used to prepare stock camelpox virus. The membranes were thawed and ground in a mortar and pestle aseptically. The mixture was freeze-thawed three times and then

sonicated using a Branson S 125 model sonifier(Heat Systems Company,N.Y). The suspension in a small vial was immersed in a beaker of ice and then a solid step horn probe used at a power setting of #5 (equivalent to 50/60 cycles per second) for 1 minute to sonicate, 8 mm beneath the surface of the suspension.This was done to completely disrupt the cells and release the camelpox virus.

73

3.4 PATHOGENICITY TRIALS OF CAMELPOX VIRUS IN LABORATORY ANIMALS

Camelpox virus obtained from Samburu was propagated to a high titre of 10^6 TCID$_{50}$/ml and inoculated in rabbits, rats and mice. Twenty animals of each species were used for inoculation. For scarification, 5 rabbits were shaved dorsally and laterally. The skin was cleaned with cotton wool soaked in 70% ethanol. Four tenfold dilutions of camelpox virus from 10^3 to 10^6 TCID$_{50}$/ml were used. Each dilution was applied in four spots. Each spot was scarified with a mounted sterile needle until hyperaemia occurred. A volume of 0.1 ml of virus suspension was then placed on each site. This was repeated for all the dilutions. This procedure was repeated on 5 rabbits using the intradermal route.

The same procedure was repeated for subcutaneous inoculation without scarification and 1ml of inoculum was used per site, in 5 rabbits. For intravenous inoculation, each of 5 Rabbits was inoculated through the jugular vein with 1ml of camelpox virus at 10^6 TCID^{50}/ml. Similar methods of inoculations were performed on rats and mice. For intradermal and scarification inoculations, 50 ul of inoculum were used on the animals. One ml of inoculum was tles used for intravenous inoculation but through the ear vein. Thermal reactions were then followed for 14 days and lesion development observed for the same period. Camelpox virus was also inoculated in 1 ml volumes into the wing of three day old chicks which had been defoliated on the inoculation site.

3.5. CAMELPOX VIRUS INACTIVATION: TRIALS FOR THE BEST
INACTIVATING AGENT

3.5.1 FORMALDEHYDE INACTIVATION

Samples of the Samburu camelpox virus at $10^5 TCID_{50}$/ml were distributed in 4 ml volumes in McCartney bottles which were placed in a waterbath at 37^0C. After allowing for equilibration, 0.5 ml of 1:2000 or 1:4000 formaldehyde was added to each sample, together with 0.5 ml of 0.4M glycine so that the final concentration of formalin was 1:20000(0.006 M formaldehyde) and 1:40000(0.003M formaldehyde) respectively, and that of glycine was 0.04M glycine. The samples were thoroughly mixed and the bottles were kept tightly closed to avoid evaporation of the reactants.

Control virus samples of 4 ml tissue culture fluid and 1 ml PBS with no formaldehyde were also kept under the same conditions. One bottle from each inactivation set was withdrawn after 15, 30, 45 min, 1, 4, 8 hr and after every 8 hr. The excess formaldehyde was neutralized with an equimolar concentration of sodium metabisulfite solution. This was accomplished by adding 0.1 ml of a 1.628% sodium metabisulfite solution to each 5 ml of the virus treated with 1:4000 formaldehyde. For 1:2000 formalin treated virus, the amount of sodium metabisulphite was doubled(Singh, 1960). The samples were then dialysed against two changes of Hanks buffered saline at 4^0 C for 48 hr and titrated for residual infectivity as before.

HYDROXYLAMINE INACTIVATION

Hydroxylamine hydrochloride($NH_2OH.HCL$) was freshly prepared as 4M and 2M solutions in 20ml volumes. The free acid was neutralized with equivalent molar concentrations and volumes of sodium hydroxide(Fellowes, 1966) and this solution was added to the virus suspension samples in bijou bottles(1.8ml) at 37^0 C to achieve final concentrations of 0.05 and 0.1 M hydroxylamine. The volume of the virus-inactivator mixture was kept constant at 2 ml Control samples with virus-maintenance medium only in the same volumes were also placed in the waterbath at 37^0 C, just like the reactants. Bottles were removed after 15, 30, 45 min 1, 8, 16, 24, 32, 40, 48, and 56 hr. The action of hydroxylamine was stopped by adding 8 ml of cold PBS(1:5)to each sample after inactivation. After dialysing twice against PBS, the samples were titrated for residual infectivity.

3.5.3 ACETYLETHYLENEIMINE INACTIVATION

Camelpox virus was placed in 2 ml volumes in McCartney bottles at 37^0C. A volume of 2ml of Acetylethyleneimine($H_2C-C-CH_3$) (AEI)at 0.1% was added to achieve a final concentration of 0,05% AEI(V/V). Control virus-maintenance medium samples in the same volumes were also placed in the waterbath at 37^0C just like the reactants. The samples were removed after 15, 30, 45min, 1 hr and after every 2 hr. Excess AEI was neutralized by adding 0.4 ml of 20% sodium

thiosulphate(w/v)solution(Brown and Crick,1959). After dialysis against PBS for 24 hr, residual infectivity was assayed.

3.6   VACCINE PREPARATION

From the inactivation results, formalin was chosen as the best one for inactivation at a dilution of 1:4000. The Samburu isolate of camelpox virus for vaccine preparation was propagated on sheep kidney up to 10^5 $TCID_{50}$/ml and was assayed on sheep kidney cells. A volume of 50 mls of the virus was mixed with Arcton 113(Trifluorotrichloroethane, I.C.I.) at a ratio of 3:1 for six hours at 4 ^{0} C for purification. Differential centrifugation at 3000 x g for 15 min was performed on the mixture after which the purified virus was mixed with formalin and glycine to make a final concentration of 1:4000 formalin and 0.04M glycine. The mixture was kept at 37^0C and inactivation performed for 48 hr. Excess formaldehyde was neutralized with an equimolar solution of sodium bisulphite and dialysed against two changes of Hanks buffered saline solution for 48 hr at 4^0C. The inactivated virus was kept at 4^0C.

3.7   VACCINE TRIALS IN RABBITS

Camelpox virus inactivated with formalin was used to vaccinate rabbits. A volume of 20 ml of camelpox virus inactivated by formalin was emulsified with 20 ml of incomplete Freund's adjuvant. Each of 10 rabbits was vaccinated through the intramuscular route

with 1ml of camelpox virus in incomplete Freund's adjuvant. The rabbits were closely checked for any reaction at the sites or temperature changes.

Sera was obtained on day 0, 6, 21 and 40 and serum neutralization test performed on them. Sera from 10 unvaccinated rabbits were similarly tested.

3.8   IMMUNISATION AND CHALLENGE OF CAMELS

After the satisfactory results in rabbits, formalin inactivated virus was considered to be the best candidate for camel vaccination. Thirty camels about 2 years old were selected. They had no previous history of either vaccination or having suffered from camelpox(Fig.18). They were shown to be sero-negative by serum neutralization test. Each of twenty camels was inoculated intramuscularly with 5 ml of formalin inactivated camelpox vaccine with incomplete adjuvant at the dorsal left hind leg site(Fig.19). Ten camels were kept as controls and were not vaccinated. On day 21 post-vaccination, all the camels (including unvaccinated controls)were challenged intradermally with four serial tenfold dilutions of virulent sheep skin adapted camelpox virus(SSV), and sheep kidney adapted camelpox virus(SKV) containing 10^2, 10^3, 10^4 and 10^5 $TCID_{50}/ml$. The (SSV) was inoculated on the inner side of the right rear leg while the (SKV) was inoculated on the inner side of the left rear leg.

The sites were cleaned with 70% ethanol. Starting with the

most rear site of each leg, the lowest dilution of virus(10^5TCID $_{50}$/ml)was inoculated as 1ml in one site by scarification and intradermal inoculation. This was done until hyperaemia but not haemorrhage was observed. This procedure was repeated for the second site 2 cm forward using 1ml of the higher dilution of the respective virus. The procedure was repeated for the third and fourth site using serial tenfold dilutions of the camelpox virus. Each of the thirty camels therefore had four sites of challenge virus on the right rear leg(SSV)and four sites on the left rear leg(SKV). The camels were restrained for this purpose with ropes and while some were standing, others were restrained on the ground.

The challenged camels were kept in an isolated camp, painted on the legs for clear marking and observed daily for any untoward reactions. The size of the reactions was measured in centimetres on day 3, 5, 10, 20, 40 and any abnormal reactions closely followed. Sera was collected from all the camels on day 0, 21, 31, 41, 61 and serum neutralization test performed on the sera.

The skin and serological responses were analyzed statistically by analysis of variance using the SPSS programme on an IBM computer.

IDENTIFICATION OF CAMELPOX VIRUS **POLYPEPTIDES**

3.9.1 VIRUS CONCENTRATION

Camelpox virus from Samburu propagated on sheep kidney cells was concentrated from one litre to 100 ml using an electronic lab assisted software hollow fibre concentration filtration unit(Bethseda lab. Inc.) using a variation of the method by Carrasco and Bravo,1986).The variables used are shown on Appendix I and the actual concentration data on Appendix II. Control tissue culture fluid with no virus but collected in the same way and from the same batch of cells was also concentrated from 500 ml to 50 ml. The procedure was repeated for both test virus and control fluids to obtain 10ml of test fluid and 5ml of control tissue culture fluid.

3.9.2 VIRUS PELLETING

The concentrated camelpox virus was then purified further on a 25% sucrose cushion using a modification of the method by Holowczak and Joklik, (1967). A preparation of 25% sucrose was prepared in phosphate buffered saline and layered slowly in 10ml polycarbonate tubes as 5ml volumes. A volume of 1ml of the concentrated camelpox virus was slowly layered on top of the sucrose solution. The tubes were then placed in position in a JA-20.1 rotor and centrifuged at 18,000 R.P.M. for two hours in a Beckman J2-21 centrifuge. The

tubes were then decanted slowly and the sucrose solution poured out. The pelleted virus was re-suspended in minimum phosphate buffered saline overnight at 4^0C. This preparation was then used for electrophoresis. The control sheep kidney tissue culture fluid with no virus was treated in a similar manner.

3.9.3   POLYACRYLAMIDE GEL ELECTROPHORESIS

The materials used for electrophoresis and their preparation are shown in Appendix III. Electrophoresis was performed in a cooled vertical slab gel apparatus(Bio-Rad Laboratories). The discontinuous electrophoresis method as described by Laemmli(1970) was used. The clean glass plates were assembled and held in place with spacers. The resolving gel was prepared in a thin walled flask by addition of most components except TEMED(Appendix III) which was added after degassing for 1 minute using a water pump. The gel was poured slowly in between the assembled glass plates. A space of about 3 cm was left above the resolving gel.The resolving gel buffer was layered on top of the gel using a syringe fitted with fine tubing. After polymerization(30 min), the assembly was tilted and the overlay poured.

The stacking gel (Appendix III) was then filled on top of the resolving gel. Immediately, the comb was inserted into the stacking gel mixture and the assembly left to polymerize. After polymerization, the comb was removed and the gel was ready for use.

The camelpox virus sample and control samples were reduced

81

and dissociated by boiling for 5 minutes in the sample buffer(Appendix IV). The samples were then loaded on the gel using a microsyringe.

The molecular weight markers were obtained from Bethseda Research laboratories. They were reconstituted (Appendix VI) and 10 ul placed on the gel (Appendix IV) using a microsyringe.

The gel was placed in the electrophoresis apparatus and electrophoresis performed at 50 mA for 6 hr at which time the tracking dye was near the edge of the gel. The gel was then recovered and stained overnight in Coomassie Blue(Appendix V). Excess stain was removed in a destaining solution(12.5% isopropanol;10% acetic acid in Tris-Hcl buffer ph 6.8) overnight. The gel was then photographed on an illuminator. The molecular weight of the viral samples were determined from the distance of migration of the polypeptides in comparison to those of the molecular markers. The molecular markers weight is shown in Appendix VI(molecular markers).

RESULTS

EPIDEMIOLOGICAL SURVEILLANCE

CAMELPOX DISEASE

Clinical cases of Camelpox were found in four herds out of forty five as shown on Table 1. Two herds of one year old camel calves were found to have camelpox in Turkana district. One herd was in the northern part of the district(Kibish), while the other herd was in the southern part of the district(Lokichar). The two regions are quite far apart(approximately 500 Km) as seen on Fig 1. The two areas were visited in the same dry season in November, and the disease condition in both places was similarly in the same acute stage. In both herds, only the calves were affected with a 100 % morbidity. Most of them were unable to feed as they had many fluid filled pustules on the lips, muzzle, and nostrils(Fig. 2). Some of the pustules had already become brown scabs. The owners reported that animals had severe swollen mouths at the onset of the disease. About one week had elapsed between the onset and our arrival. Of the thirty six scabs from infected camels in Turkana district, thirty of them revealed pox viruses after negative staining(Fig.3). Camelpox virus was isolated (on sheep kidney cells) from twenty cases in Kibish and ten cases in Lokichar. Camelpox virus was also recovered (on embryonated eggs) from 29 scabs.

83

Camelpox was also diagnosed in two adult herds in Samburu from camels which had been brought from Somalia and Galana ranches(Fig.4). In the camels from Somalia, a herd of 45 camels brought to Wamba, Samburu district and ranging in age between 3 and 6 years, came down with serious camelpox infection two weeks after arrival. The camels had arrived three weeks after the end of the short rains. All the camels had many pustules on the lips, muzzle and nostrils(Fig 5). The eyes were discharging in most of them and all of them had swollen cervical lymph nodes. Fifteen of them had swollen and oedematous skin on the jaws and neck. Twenty samples of scabs obtained from the camels had camelpox by direct electron microscopy. Camelpox virus was isolated from eighteen of the samples by propagation in sheep kidney cells and from twenty samples by propagation on embryonated eggs(Table 1).

A similar pattern was found in 60 camels brought from Galana ranch. They were all mature camels of five to seven years and had been transported on road from Galana ranch to Kisima, Samburu district. The owner of the animals said none of the animals had had camelpox at Galana ranch and he had not experienced camelpox in all his animals since he started camel rearing thirty years ago in his Galana ranch. All the camels came down with a severe camelpox infection two weeks after arrival. The camels had pustules and scabs on their lips, muzzle and nostril. Most of them had discharging eyes and thirty of them had severely swollen mandible and neck regions(Fig.5). Most of the camels were unable to feed properly for about two weeks and had lost weight considerably. In

twenty scabs which were obtained, camelpox virus was observed in 17 cases by negative electron microscopy From 15 of 20 scabs, camelpox was isolated on sheep kidney cells, while on 17 of the scabs were positive for virus on egg inoculation.

4.1.2 SEROLOGICAL PATTERN.

The neutralization results are as shown in Table 2.Most of the camels in many camel rearing areas were sero-positive for camelpox virus.The sick camels, however, had much higher titres than normal camels(Table 3).

4.2 CAMEL CONTAGIOUS ECTHYMA

In the Turkana district investigation, four herds of camel calves with contagious ecthyma infection were found.The outbreaks were in four different areas as indicated in Table 4 and Fig.1.

In the four outbreaks, goat kids in the same households also had an infection with a parapox virus. Although the outbreaks were in different areas, the four herds had similar identical lesions all at approximately the same stage of infection. Most of the camel calves had an acute infection and had pustules on their lips, muzzle and nostrils. Most of the camel calves were therefore unable to feed properly. Some of the lesions had progressed to scab formation and these were hard and brownish in colour(Fig.6). Almost all the calves had severe secondary infection with purulent

85

material evident under the scabs. Many of the camel calves had swollen mandibular lymph nodes which some of the pastoralists had tried to manage by branding.

In all the four herds involved, although all camels were normally herded together, no adults had any sign of infection. The parapox virus was demonstrated by negative electron microscopy from the four herds as shown on Fig.7.

The goat kids which were involved also had similar lesions to those in the camels (Fig.8). Most of them were also in the acute stages with pustules in the nostrils, muzzle and lips. The lesions, however seemed to be more severe in the goats than in the camels. They also had more secondary infections than the camels. Most of the pustules had thick yellowish exudate and some were so severe that the kids had problems in breathing and also had mucoid discharges from the nostrils. Most of these scabs on removal revealed raw, bleeding skin with thick encrustations of yellowish discharge. In two herds in Turkana, each of two goat dams was found to have the same pustular lesions on teats. One dam had two teats with pustules, while in the other dam only one teat had pustules.

4.3   **PROPAGATION OF CAMELPOX VIRUS**

4.3.1   **PROPAGATION OF CAMELPOX VIRUS IN EMBRYONATED EGGS**

White pock lesions with a diameter of 2-3 mm were obtained regularly, when embryonated eggs were infected with camelpox

virus(Fig.9). At the site of inoculation, the pock lesions were fused into plaques with a diameter varying from 0.5 cm to 2 cm. depending on the size of the inoculum. These areas were slightly raised from the surface and were surrounded by an oedematous membrane with slight haemorrhage. The distinct pock lesions found in the rest of the membrane were variable in shape but mostly round or oval with a raised centre.

4.3.2 PROPAGATION OF CAMELPOX VIRUS IN CONTINUOUS CELL LINES

Camelpox virus was propagated on BHK-21 cells and Vero continuous cell lines. In the continuous cell lines, the effect of camelpox virus infection was observed 4 days(96 hours) post-infection. The first sign of infection was cell rounding in various foci and cells with increased refractivity. After 6 days, this had progressed to start showing syncytia formation with strands. The maximum effects of camelpox infection were observed on the seventh day when cell death with detachment of cells became visible. Camelpox virus propagated in these continuous cell lines were titrated on homologous cells, and they consistently produced low titres of 10^3 TCID $_{50}$/ml.

4.3.3 PROPAGATION OF CAMELPOX VIRUS IN PRIMARY CELLS

The primary cells on which camelpox was propagated were calf testis, calf thyroid and calf lungs; Sheep testis, sheep skin,

sheep kidney and sheep lungs.The development of cytopathic effects on these cells was similar. The first cytopathic effects were visible two days(48 hours) post-infection. There was cell rounding in various foci. Cells in these foci had increased refractivity. Giant cell formation was visible after 4 days(96 hours) and reached a maximum in 6 days(144 hours). These giant syncytia were formed by about 20 cells and were quite characteristic with strand formation and maximum refractivity. By the seventh and eighth day, many of the affected foci had detached from the glass and peeled of. Sheep kidney cells appeared the most convenient for the propagation of camelpox virus and titres of 10^5 $TCID_{50}$/ml were consistently obtained after titration in homologous cells. They were also easily passaged. On the contrary, some cells like sheep skin and calf thyroid were tedious to prepare and sheep skin particularly, was very difficult to passage. The titres of virus obtained were however approximately the same. Fig.10 indicates the appearance of the cytopathic effects in sheep kidney cells and Fig.11 indicates control sheep kidney cells which have not been inoculated with virus. Fig 12 indicates the pathogenic effects of camelpox virus on sheep skin cells while Fig. 13 shows sheep skin cells where there was no virus infection and the cells formed a uniform monolayer. Inclusion bodies of the B type were commonly found. They are shown in Fig. 14.

4.3.4 PLAQUE FORMATION

Distinct plaques were formed measuring 2 to 3 mm in diameter with rough edges visible with the naked eye. Under the microscope, these plaques were made of areas with extensive cell death and many syncytia formations(Fig.15). There were many strands in between the cells but the central area of the plaque was mainly empty.

4.3.5 PATHOGENICITY

Camelpox virus was not pathogenic in any of the inoculated laboratory animals.Their temperatures remained normal and there were no pox lesions developing.

4.4 VIRUS INACTIVATION

4.4.1 FORMALIN INACTIVATION

The inactivation of camelpox virus with formalin is depicted on Fig.(16). The 1:2000 concentration of formalin had reduced the original 10^5 TCID$_{50}$/ml of camelpox virus to $10^{1.5}$ by 24 hours at 37^0C and with 1:4000 formalin concentration, the camelpox virus had been reduced to $10^{0.8}$TCID$_{50}$/ml by 36 hours. Complete inactivation occurred after 36 hr and 48 hr respectively with 1:2000 and 1:4000 formalin preparation.

4.4.2   HYDROXYLAMINE INACTIVATION

The inactivation of camelpox virus with 0.5M and 0.1M hydroxylamine is depicted on Fig.17. Camelpox virus had been completely inactivated in 24 and 36 hr respectively by 0.5M and 0.1 M hydroxylamine.

4.4.3   ACETYLETHYLENEIMINE INACTIVATION

The inactivation of camelpox virus with 0.05% acetyl-ethyleneimine is depicted in Fig.18. The inactivation followed a first order kinetics and virus was inactivated by 10 hours.

4.5   VACCINE TRIALS IN RABBITS.

The rabbits had no untoward signs on their challenge sites.Their antibody profile is as depicted in Fig.19. Antibody levels rose steadily after vaccination and peaked at 21 days before levelling off. Their temperatures remained normal.

4.6   CAMEL RESPONSE TO VACCINATION

4.6.1   SKIN RESPONSE TO CHALLENGE BY CAMELS
The experimental camels used for this experiment are shown on Fig. 20 and Fig. 21 shows the manual restraint of camels. All camels including the unvaccinated and vaccinated camels had lesions

on day three post-infection as indicated on Fig.22,23 and 24. The skin lesions were however mild with slight elevation and showing hyperaemia.

In the 20 vaccinated camels, most of the low dilutions of challenge(10^5 TCID $_{50}$/ml)virus had many lesions while the second low dilution (10^4 TCID $_{50}$/ml) had few lesions.There were very few lesions on the sites inoculated with the third(10^3 TCID$_{50}$/ml) and fourth dilutions(10^2 TCID $_{50}$/ml) as indicated on Fig.24. The response from sheep skin adapted camelpox virus was significantly higher than that of sheep kidney adapted camelpox virus($p<.05$).

By day 5, however, all the lesions on the control animals had resolved to leave healed skin. Five camels in the control group developed abscesses later on as shown in Table 5 and Fig.25 and staphylococcus aureus was isolated from the abscesses.

In the 10 test camels which had not been vaccinated, there were small lesions initially but these increased in size upto day 10 and then decreased in size and had healed by day 20(Fig.26 and 27). Many lesions developed through the typical stages of pox lesions, that is papule, vesicle, pustule and scab formation (Fig 22,23,28,29 and 30.). Fig.22 shows the response of camels inoculated with skin adapted camelpox virus while Fig. 23 shows the response of camels inoculated with kidney adapted camelpox virus five days post inoculation. Fig.28 shows the response of camels inoculated with sheep kidney adapted camelpox virus while Fig.29 shows the response of camels inoculated with sheep skin adapted camelpox virus ten days post inoculation. There was central

necrosis in many of the lesions. Fig 30 shows scab formation.

On all the days of measurement, for the test group, there were no significant differences between the responses of sheep skin adapted virus and sheep kidney adapted virus(p<0.05). There was a linear dose response curve for both the test and control group. This is shown on Fig.31 where sheep skin adapted camelpox virus was used for challenge and on Fig.32 where sheep kidney adapted camelpox virus was used for challenge. In the test group, one camel developed an abscess from which haemolytic and non-haemolytic *Staphylococcus aureus* were isolated.

4.6.2   NEUTRALIZATION TESTS

The mean antibody profile of the camelpox vaccinated camels is depicted in Fig.33. The vaccinated camels developed antibodies with the titres peaking on day 40. The test camels had no antibodies until after challenge on day 21. The antibody titres of the vaccinated camels were significantly higher than those of the non-vaccinated camels(p<0.05) 31 days post-vaccination. This was 10 days post challenge when there was significant skin immunity as tested by dermal scarification.

4.7   CAMELPOX POLYPEPTIDES

The camelpox polypeptides migrated in proportion to their molecular weights as depicted on Fig.34. Four distinct camelpox

polypeptides absent from virus-free cell filtrates were observed. Their molecular weights were determined by plotting log $_{10}$ molecular mass against the distance migrated by marker polypeptides (Fig.35). The molecular weight of the five viral polypeptides is shown on Table 6.

TABLE 1: CLINICAL MANIFESTATION OF CAMELPOX IN THE HERDS OF CAMELS SURVEYED AND VIRUS ISOLATION FROM THE LESIONS

parameter	Location and number of cases			
	Kibish*	Lokichar*	Wamba**	Kisima**
Age	2/3-1yr	2/3-1yr	3-6yr	5-7yr
No affected	24/40	12/30	45/45	60/60
Samples	24	12	30	20
EM +ve	20/24	10/12	20/30	17/20
Egg +ve	19/24	10/12	20/30	17/20
Cell +ve	20/24	10/12	18/30	15/20

Note:

* In Turkana district, both herds with facial pustules

Wamba**   - Samburu herd from Somalia with facial pustules
            and neck oedema

Kisima**   - Samburu herd from Galana ranch with facial
            pustules and   neck   oedema

EM' +ve - camelpox positive by Electron microscopy
Cell +ve - camelpox positive by propagation in cells
Egg +ve - camelpox positive by propagation in
            embryonated eggs

TABLE 2: CAMELPOX ANTIBODY TITRE IN CLINICALLY SICK CAMELS FROM DIFFERENT AREAS

Area	Total sera	Antibody neutralization titre				
		160	320	640	1280	2560
Turkana	30	2	4	10	8	6
Somalia	30	1	4	5	18	2
Galana	35	0	5	6	19	5

Note: The numbers in the table indicate the number of camels with the particular antibody titre(reciprocal) indicated above

TABLE 3: CAMELPOX ANTIBODY TITRES IN CLINICALLY NORMAL CAMELS FROM DIFFERENT AREAS OF RIFT VALLEY PROVINCE

Area	Total camels	Antibody neutralization titre					
		40	80	160	320	640	1280
Turkana	100	12	38	22	8	20	0
Samburu	40	5	19	8	8	0	0
Laikipia	20	10	5	5	0	0	0

Note : The numbers in the table indicate the number
of camels with the particular antibody titre (reciprocal)
indicated above

TABLE 4 : POSITIVE CASES OF CAMEL CONTAGIOUS ECTHYMA IN TURKANA DISTRICT

Locality	Animals affected					
	Camels			Goats	
	age(m)	No affected	EM+	No affected	EM+
Kakuma	8-12	12/40	10/12	20/100	18/20
Kaloko	8-18	20/60	7/20	5/20	5/5
Lokori	8-12	30/80	20/30	10/50	7/10
Lokitaung	6	5/20	5/5	20/80	16/20
Total		67/200	52/67	55/250	46/55

Note:EM +ve = Positive by Electron
microscopy.

TABLE 5 : ABSCESS DEVELOPMENT ON CHALLENGE SITES ON VACCINATED AND UNVACCINATED CAMELS CHALLENGED ON DAY 1 USING 4 SERIAL LOG DOSES OF SHEEP KIDNEY ADAPTED CAMELPOX VIRUS(SK) AND SHEEP SKIN ADAPTED CAMELPOX VIRUS(SS).

CAMEL NO.	DAY SEEN	SIZE(cm)	SITE	DAY HEALED	ORGANISM OBTAINED
CONTROL(VACC)					
245	20	6	-1(SK) *	40	S.aureus(H+NH)***
251	20	5	-3(SS)**	40	S.aureus(H+NH)***
258	40	4	-4(SS)**	60	S.aureus(H+NH)***
267	40	6	-4(SK)*	60	S.aureus(H+NH)***
456	40	4	-3(SS)**	60	S.aureus(H+NH)***
TEST(UNVACC)					
252	40	6	-4(SS)**	60	S.aureus(H+NH)***

Note:

SK*   = Sheep kidney adapted camelpox virus
SS**   = Sheep skin adapted camelpox virus

H ***   = Haemolytic organism
NH ***   = Non-haemolytic organism

TABLE 6: THE MOLECULAR WEIGHTS OF CAMELPOX VIRUS POLYPEPTIDES

AS DETERMINED BY ELECTROPHORESIS AND COMPARED WITH THE STANDARDS

polypeptides	Distance (mm)	Molecular weight
standards		
m	8	97,400
n	23	68,000
o	32	43,000
p	62	29,000
q	77	18,400
r	90	14,300
viral		
1	20	71,753
2	30	62,426
3	62	32,581
4	70	25,120
5	90	6,466

Note: m-r   =   standard markers polypeptides
      1-5   =   Viral polypeptides
      Distance(mm)= Distance migrated by polypeptides

Fig. 1   MAP OF TURKANA DISTRICT

Fig. 1 MAP OF TURKANA DISTRICT
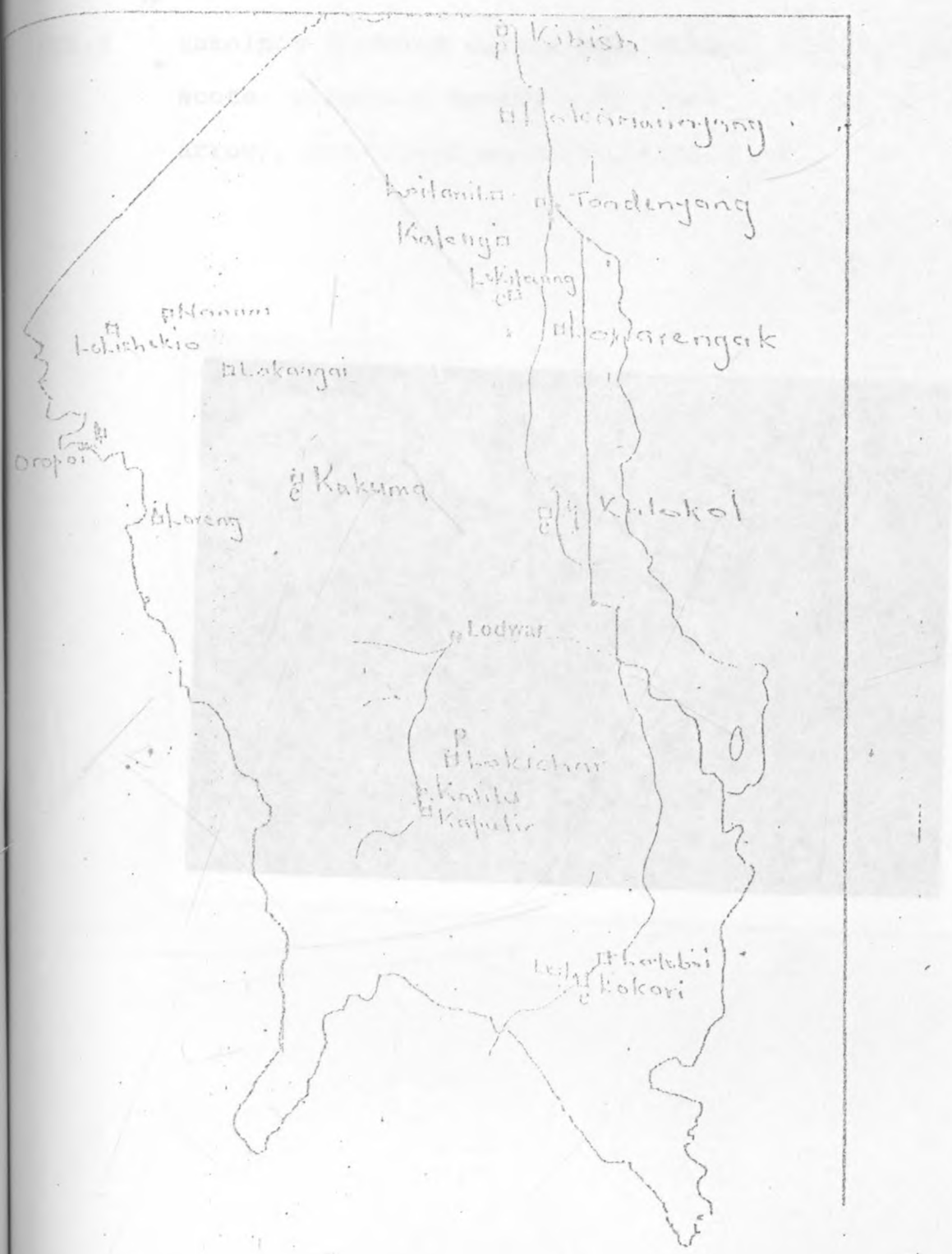
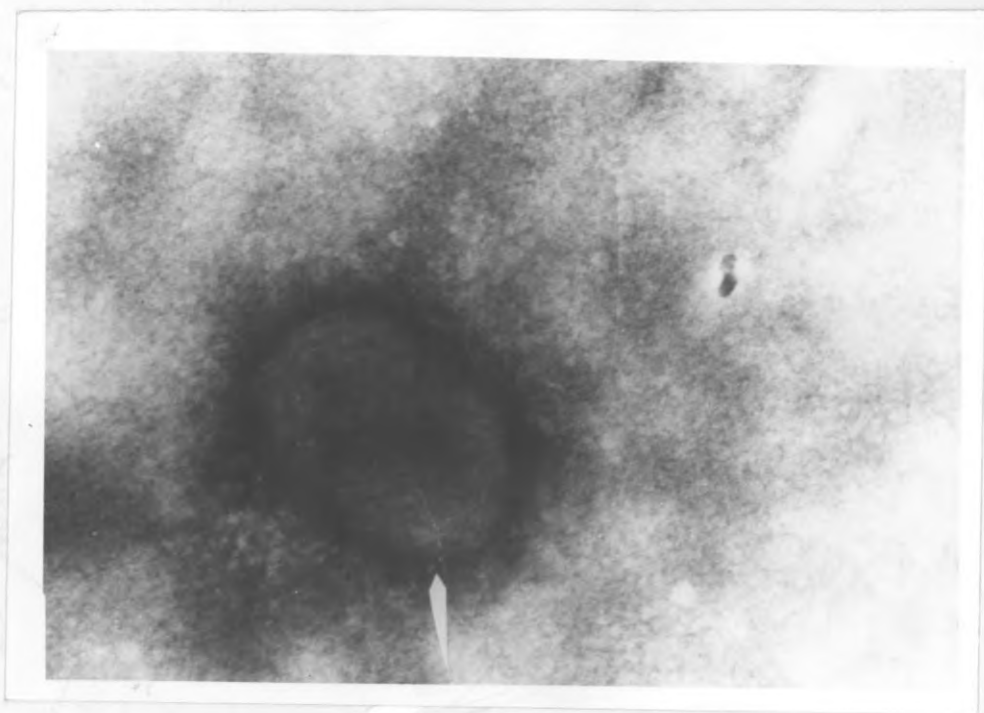e — Areas where camel contagious ecthyma was found

P — Areas where camelpox was found

FIG.2   Camelpox infected calves from Lokori, Turkana with an
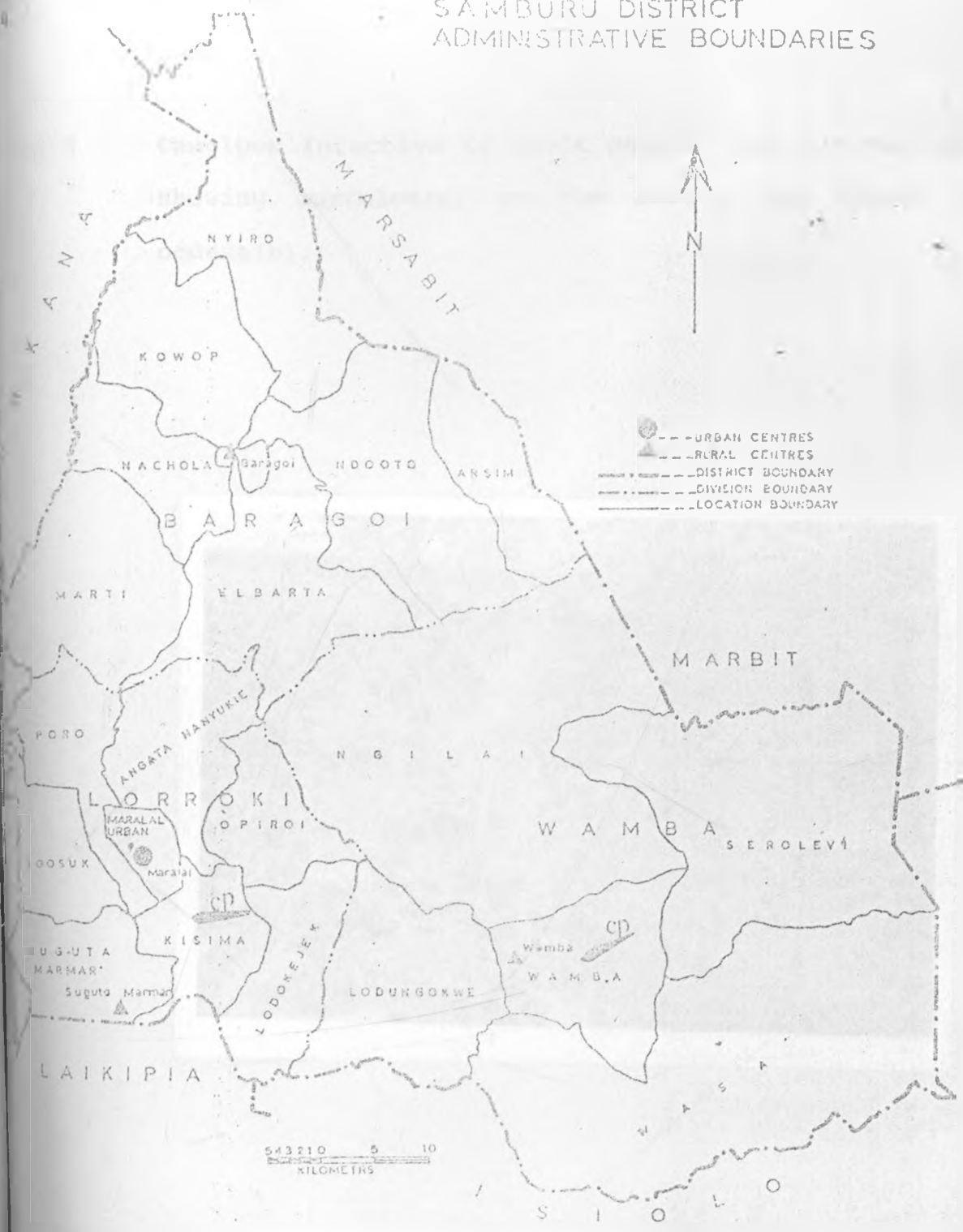   acute   infection having many pustules on the lips(black
         arrow), muzzle and nostrils(white arrow).

FIG. 3   Electron micrograph of a camelpox virus(arrow;X60,000)

cp   Areas where camelpox was found.

Fig.5   Camelpox infection in adult camels from Kisima,Samburu
         showing pustules(a) on the muzzle and severe neck
         oedema(b).

FIG 6   Clinical contagious ecthyma virus infection in camel
         calves from Turkana having many pustules(a) on the lips
         and showing lymph node branding by the owners(b).

G.7   Electron micrograph of camel contagious ecthyma
        virus(260 X150nm)from camel calves in Turkana(X60,000;
        arrow).
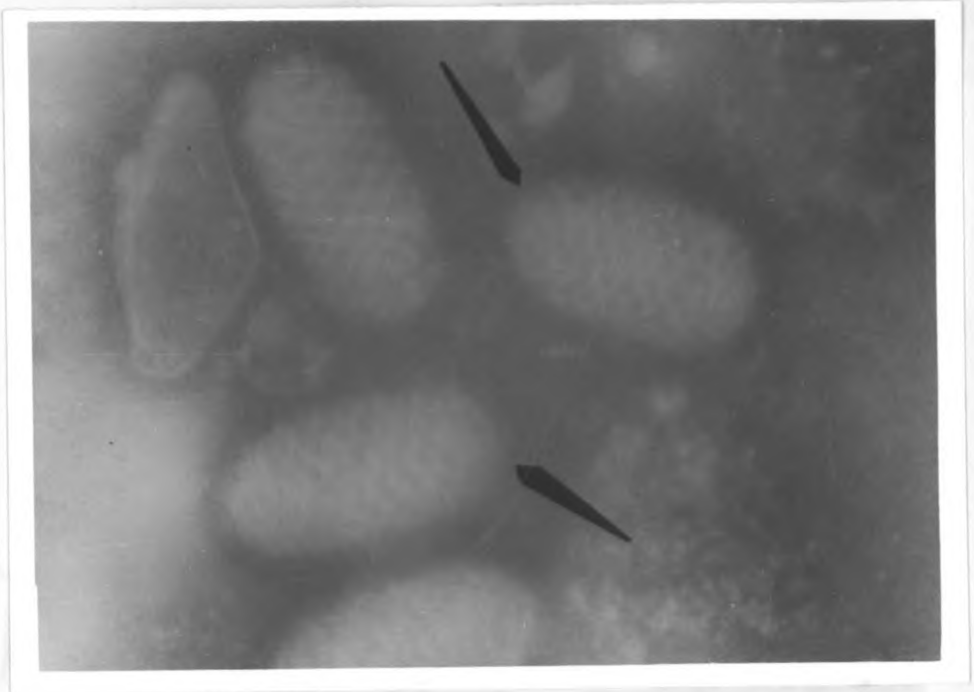
FIG 8   Clinical infection of a goat kid with contagious ecthyma virus in Turkana having severe pustules on the muzzle (arrow).

FIG 9   Camelpox virus propagated on chorio-allantoic membrane
         showing white pock lesions(arrows) of approximately 2-
         3mm in diameter.

FIG.10   Camelpox virus propagated on sheep kidney cells for 5
         days after inoculation at a multiplicity of infection
         (m.o.i. of 20) showing syncytia   formation(arrows;
         X1000).
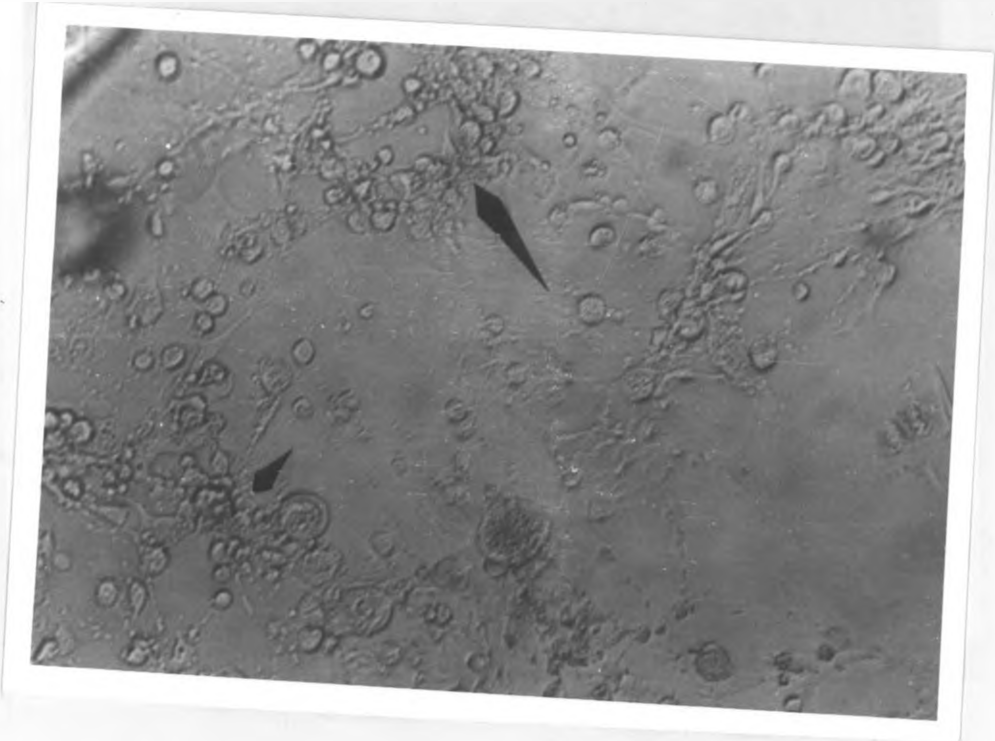
Fig.11   Control sheep kidney cells which have not been infected
           with virus showing the smooth monolayer (X400).
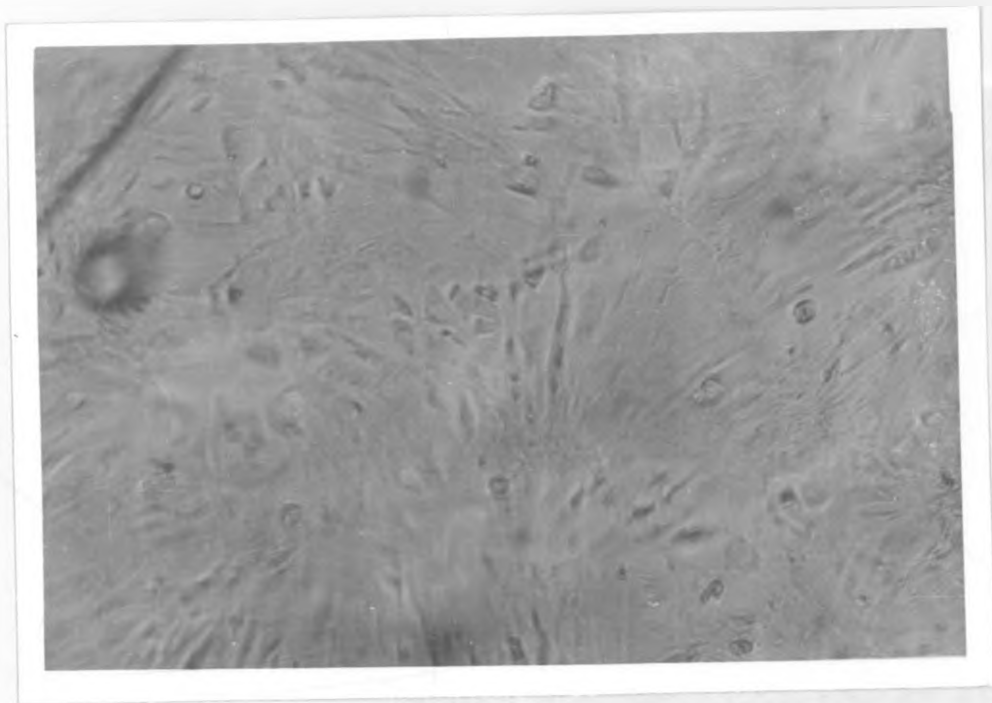
FIG.12 Camelpox virus propagated on sheep skin cells for 5 days after infection (m.o.i. of 20) showing syncytia formation(arrows; X400).

Fig.13   Control sheep skin cells which have not been infected
          showing the thin spindle shaped cells(X400).
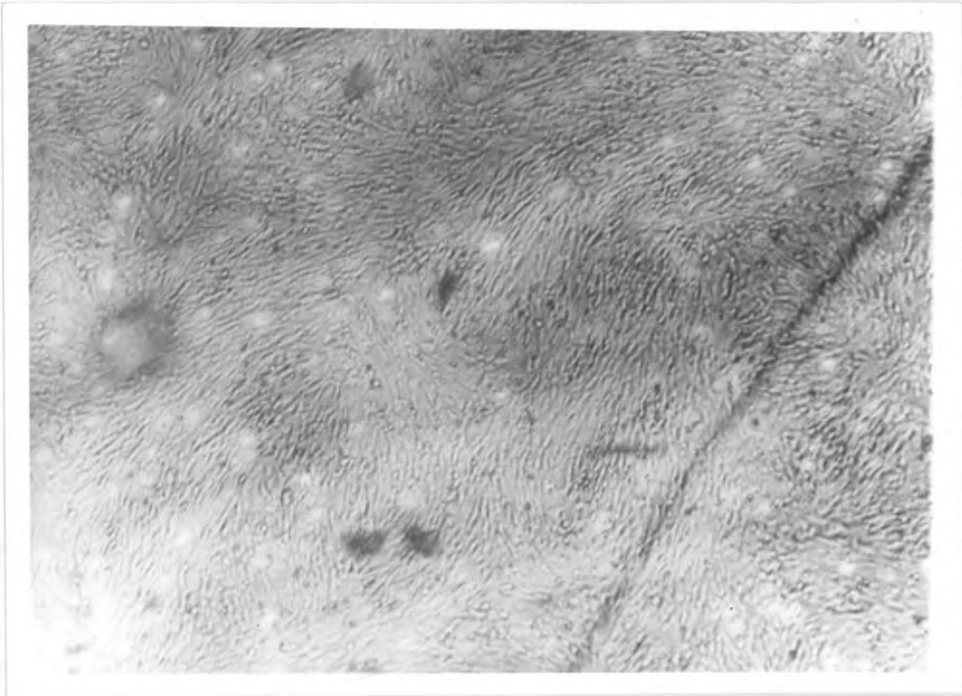
FIG.14   Sheep kidney cells which have been infected with camelpox
          virus(m.o.i of 20), showing the characteristic "Halo"
          shape(arrows) of inclusion bodies(H&E; X1000).

FIG.15   Sheep kidney cells which have been infected with
           camelpox virus (m.o.i of 20), then fixed and stained
           with crystal violet showing syncytia formation(A) and the
           plaque(B).
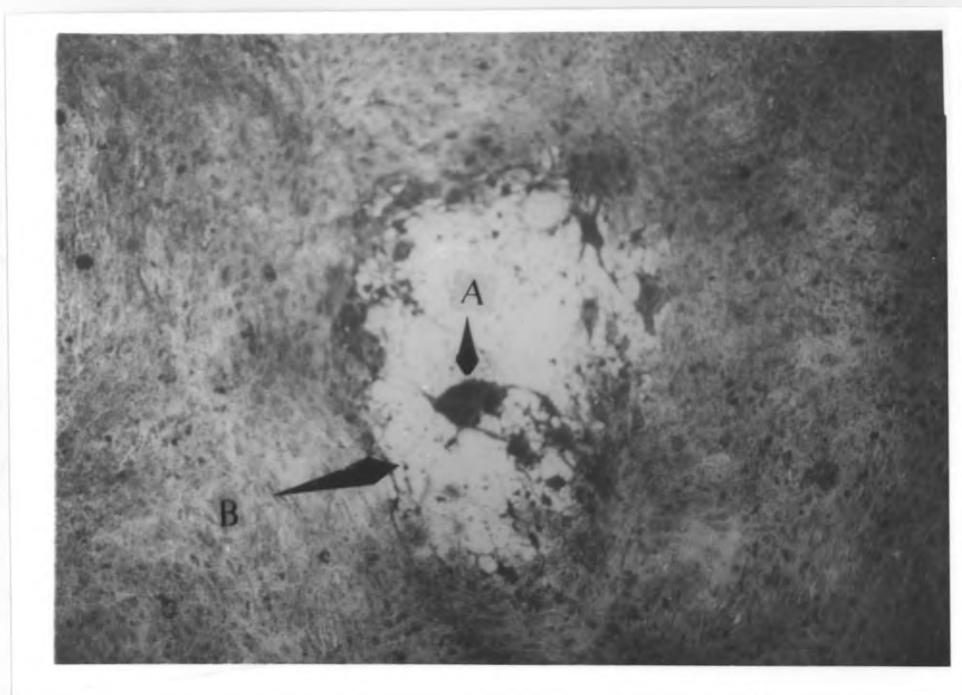
Fig.16
The inactivation time of camelpox virus with 1:2000 formalin and 1:4000 formalin

Legend:
- ○········○ 1:2000 formalin
- ●– – ● 1:4000 formalin

Y-axis: Log TCID 50/ ml
X-axis: Hours of Inactivation
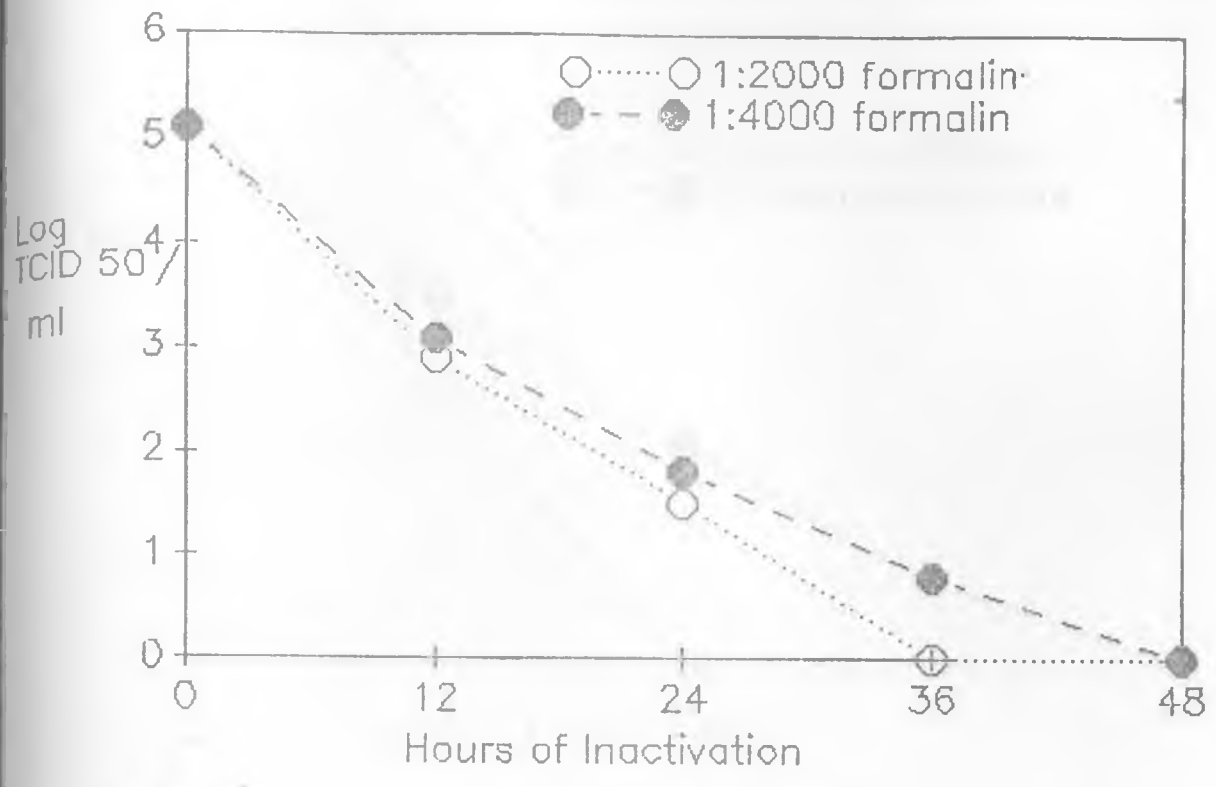
Fig.17
The inactivation time of camelpox virus using 0.5M
Hydroxylamine Hydrochloride

Legend:
- O——O 0.5Mhydroxylamine
- ●——● 0.1Mhydroxylamine

Y-axis: Log TCID 50/ml
X-axis: Hours of Inactivation
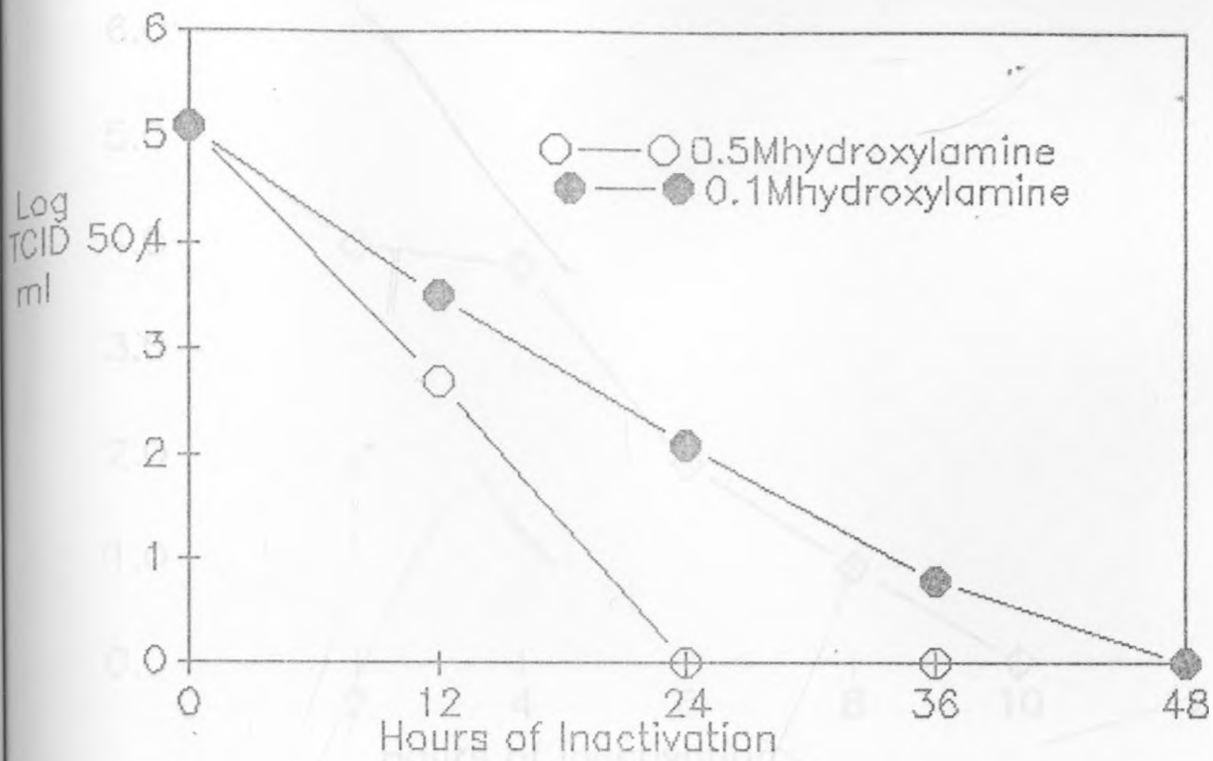
Fig. 18
The inactivation time of camelpox virus using 0.05%
Acetylethyleneimine
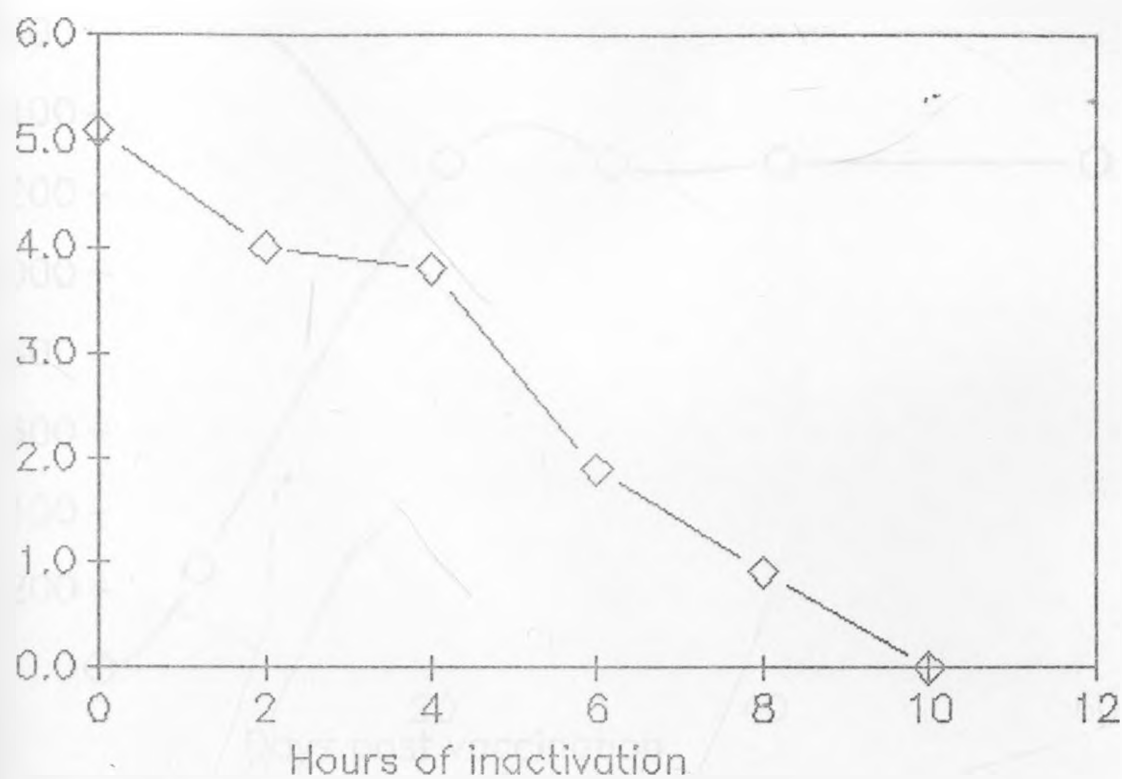
Fig. 19
The serological response of rabbits vaccinated with a camelpox
virus inactivated with formalin
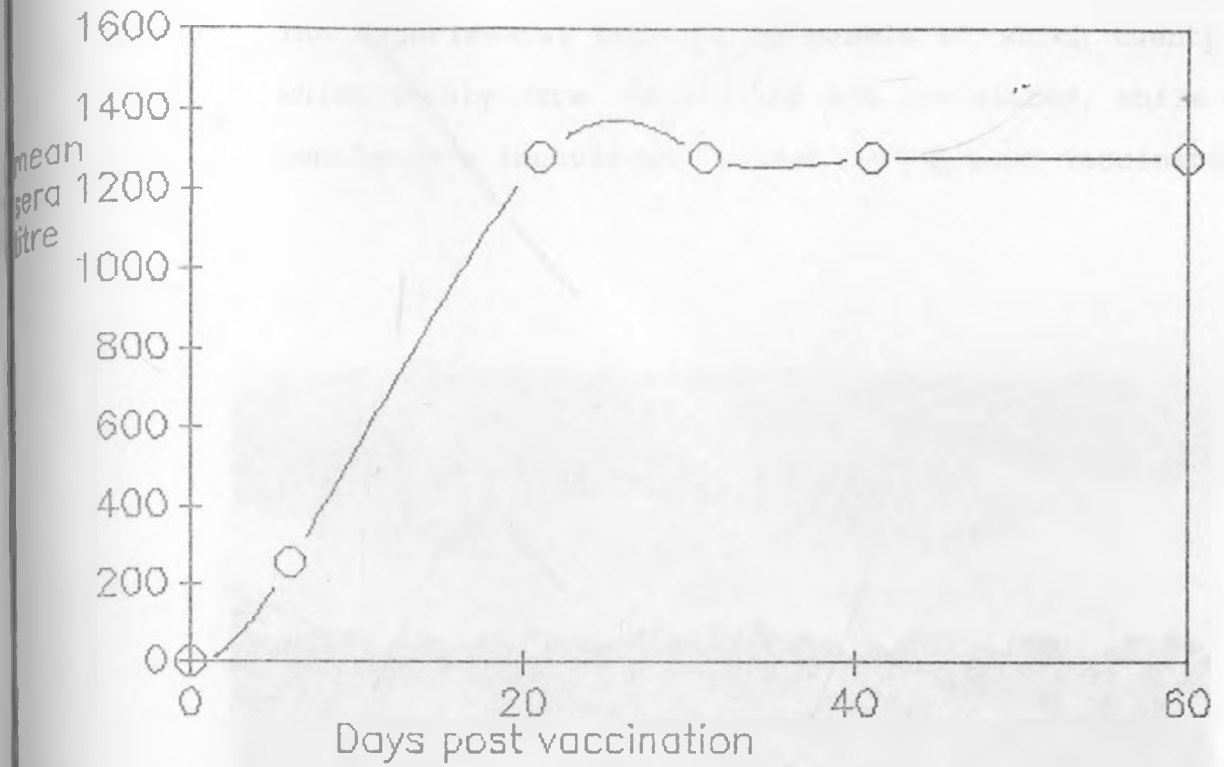
FIG.20   The experimental herd of 30 camels of which twenty of
          which twenty were vaccinated and inoculated, while ten
          camels were inoculated without having been vaccinated.

FIG.21 The manual restraint of camels during bleeding.

FIG.22   The skin reaction of test camels(non-vaccinated)five
days post inoculation with skin adapted camelpox
virus(right hind leg).

Virus titre

A - 10^3 TCID 50/ml

B - 10^4 TCID 50/ml

C - 10^5 TCID 50/ml

Fig.23 The skin reaction of test camels(non-vaccinated)five days post inoculation with sheep kidney adapted camelpox virus(left hind leg).

Virus titre

A - 10^3 TCID 50/ml

B - 10^4 TCID 50/ml

C - 10^5 TCID 50/ml

FIG.24 The mild skin reaction of control (vaccinated) camels
five days after inoculation with sheep kidney adapted
camelpox virus.

Virus titre

A - 10^3 TCID 50/ml

B - 10^4 TCID 50/ml

C - 10^5 TCID 50/ml

FIG.25   Abscess development in some vaccinated camels 40 days
          after inoculation with sheep kidney propagated camelpox
          virus.

development of skin lesions after inoculation with sheep kidney
propagated camelpox virus on unvaccinated camels
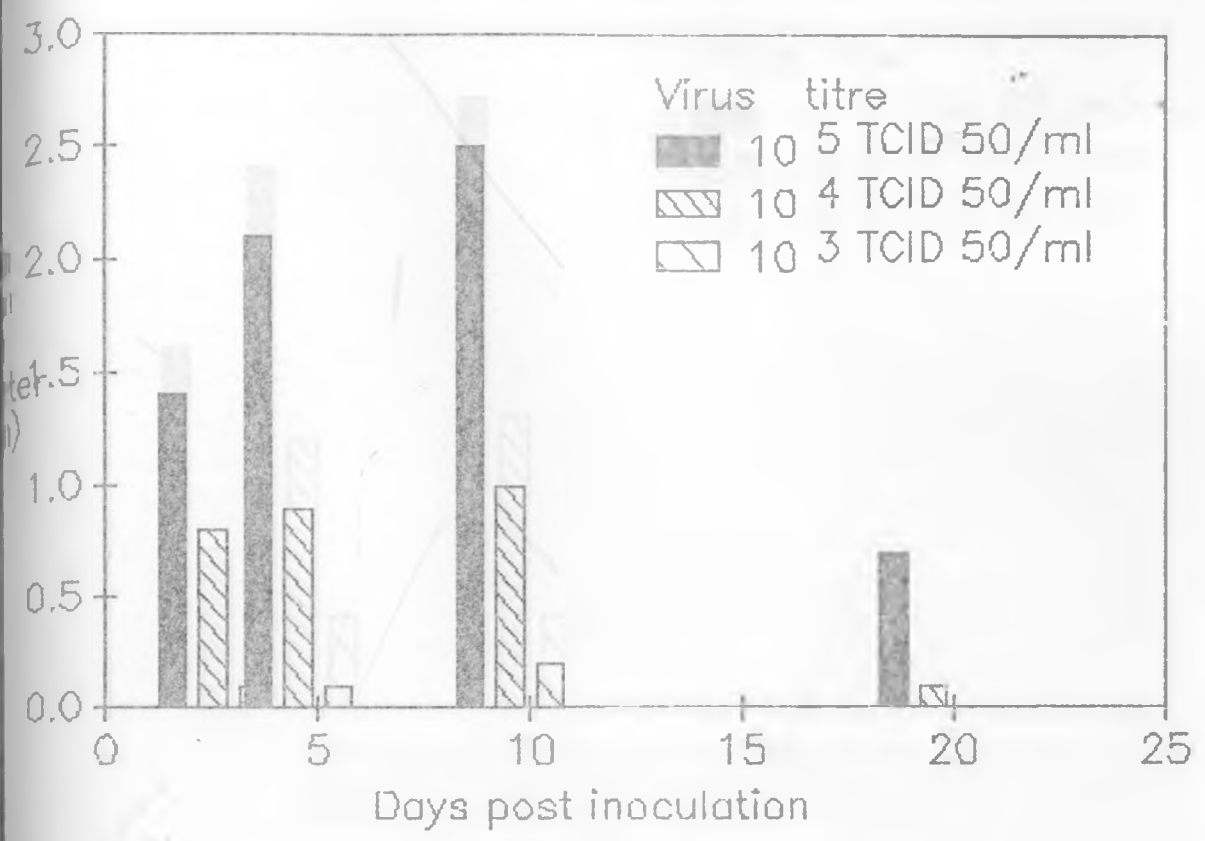
mean skin lesion sizes after inoculation with sheep skin
propagated camelpox virus on unvaccinated camels
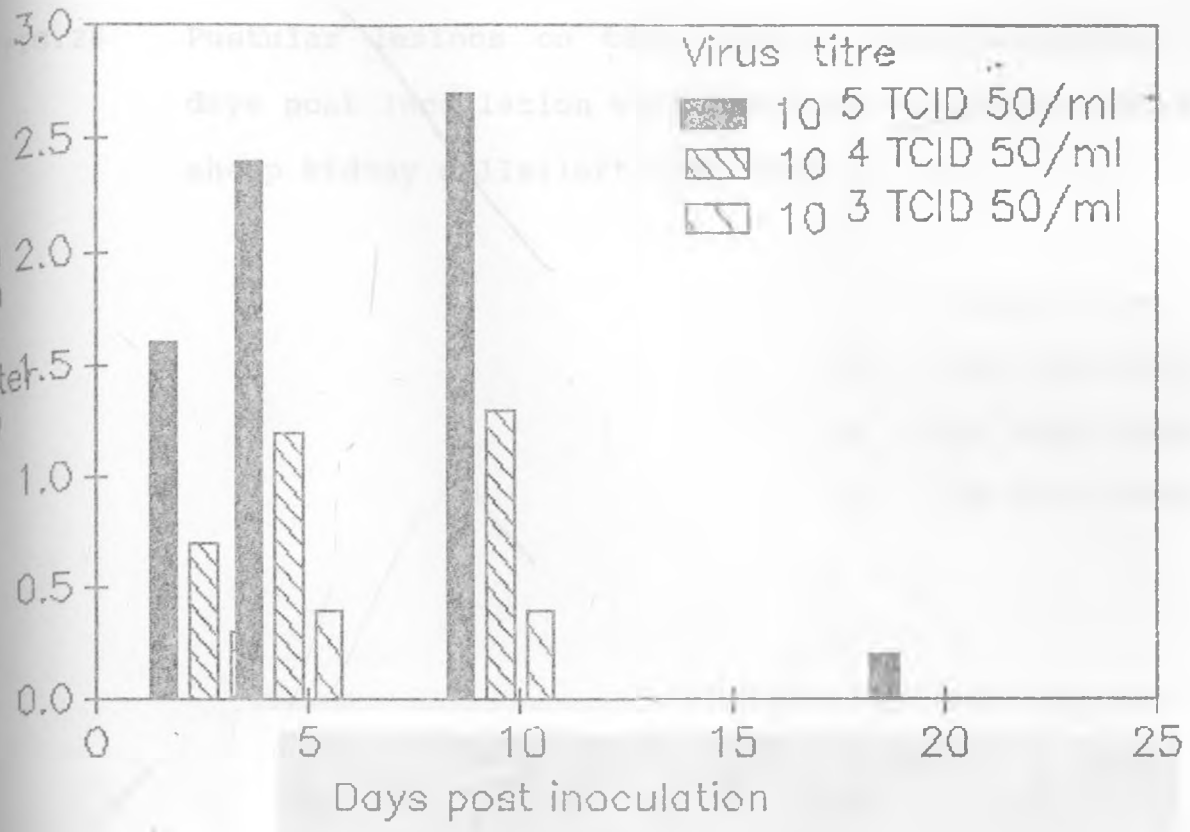
FIG.28   Pustular lesions on test camels (non-vaccinated) ten
          days post inoculation with camelpox virus propagated on
          sheep kidney cells(left hind leg).

                                              Virus titre
                                    A - 10^3 TCID 50/ml
                                    B - 10^4 TCID 50/ml
                                    C - 10^5 TCID 50/ml

Fig. 29   Pustular lesions on test camels(non-vaccinated)ten days
           post inoculation with camelpox virus propagated on sheep
           skin cells(right hind leg).

                                    Virus titre
                              A - 10^3 TCID 50/ml
                              B - 10^4 TCID 50/ml
                              C - 10^5 TCID 50/ml

Fig. 30   Scab development and peeling off of skin lesions in
           test camels(non-vaccinated)20 days post inoculation with
           camelpox virus propagated on sheep skin(left hind leg).

                              Virus titre
                   A   - 10^3 TCID 50/ml
                   B   - 10^4 TCID 50/ml
                   C   - 10^5 TCID 50/ml
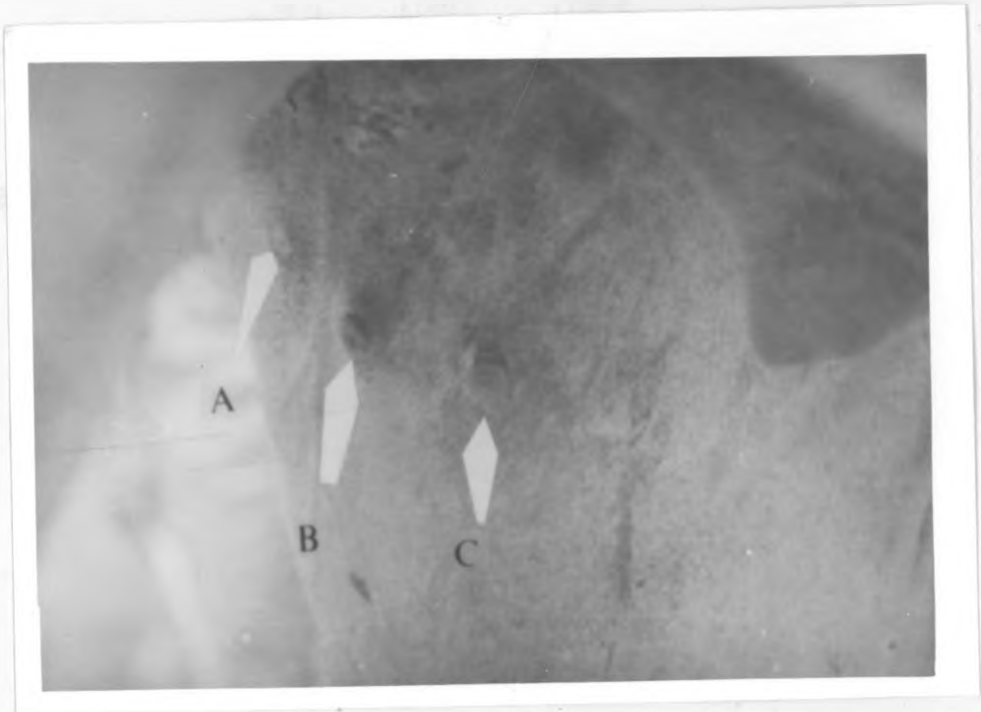
skin lesion sizes of unvaccinated camels after inoculation with
erent doses of camelpox virus propagated on sheep kidney cells
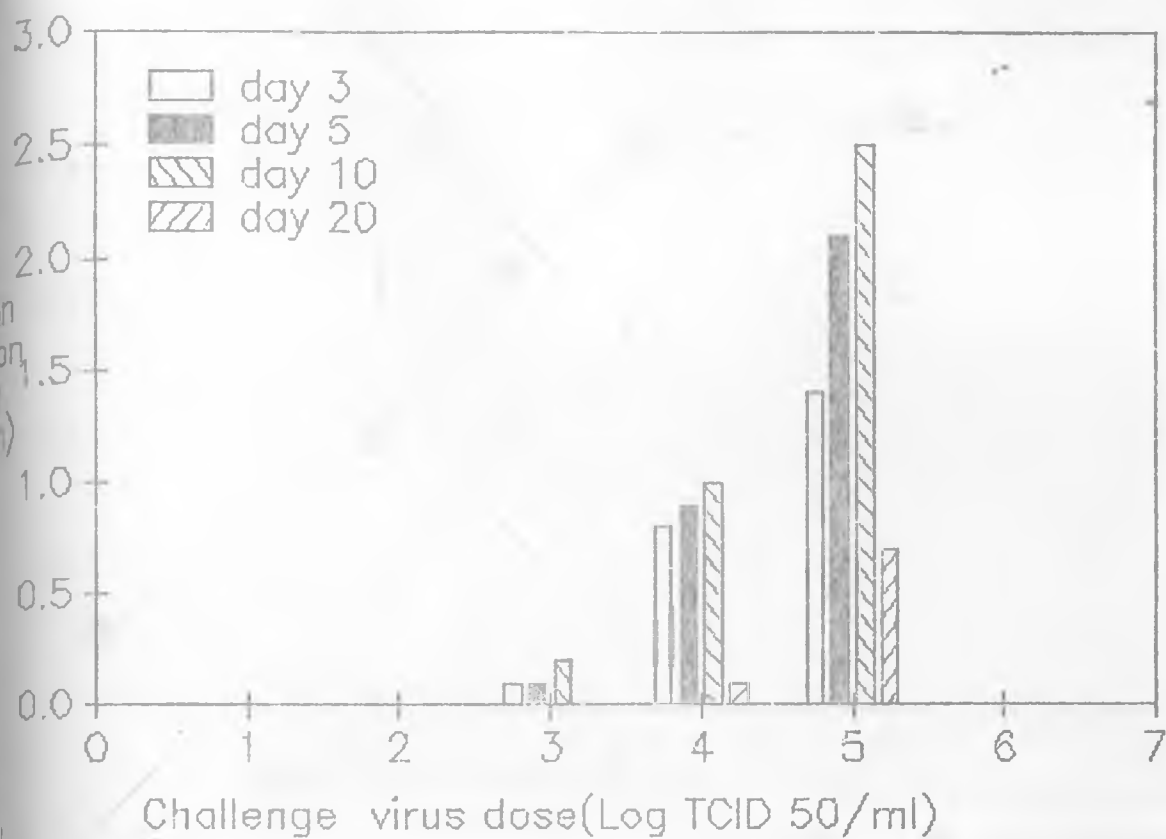
neutralization activity of sera from unvaccinated and
cinated camels before and after challenge
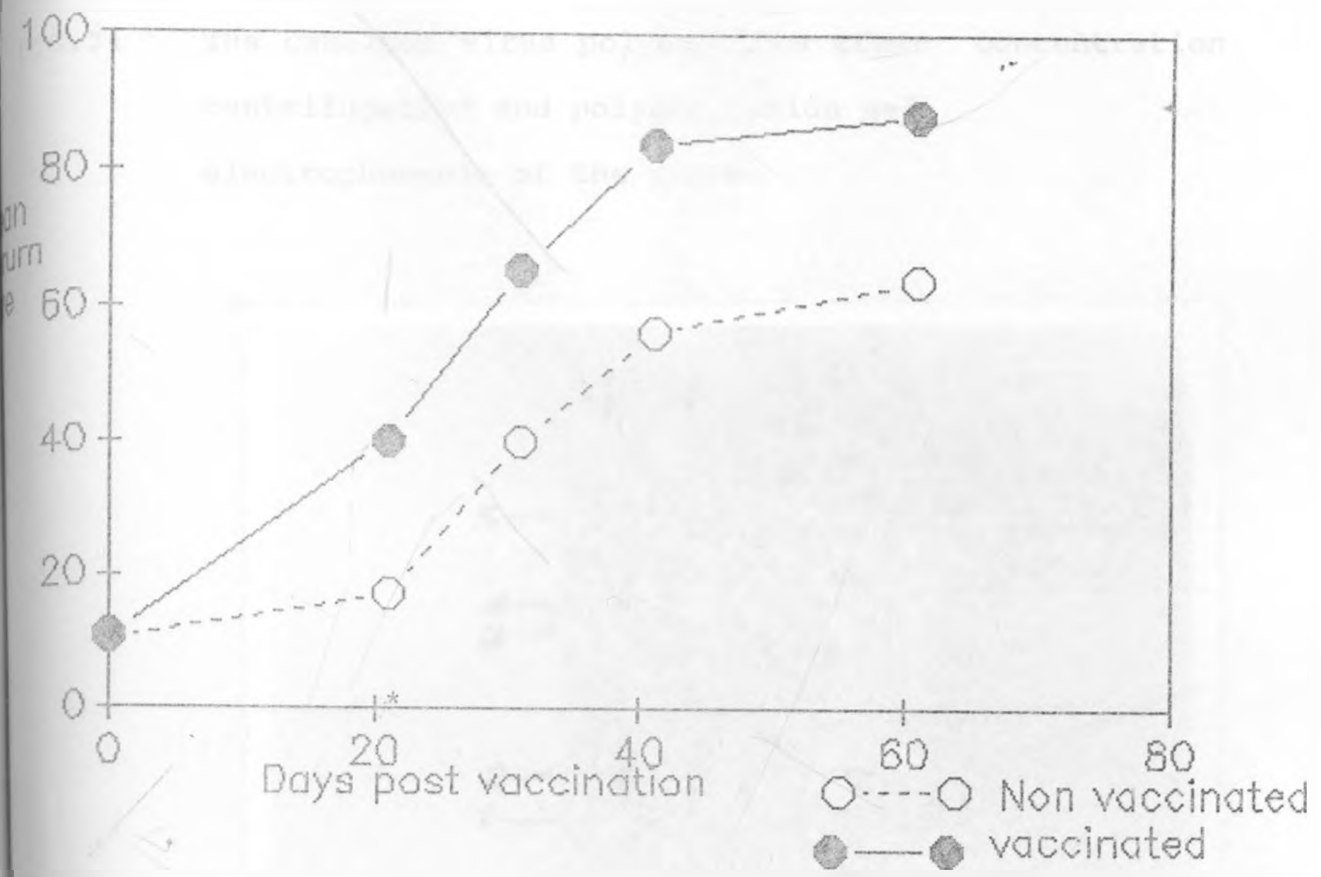
Note : * = Day of challenge.

FIG.34   The camelpox virus polypeptides after concentration
          centrifugation and polyacrylamide gel
          electrophoresis of the virus.
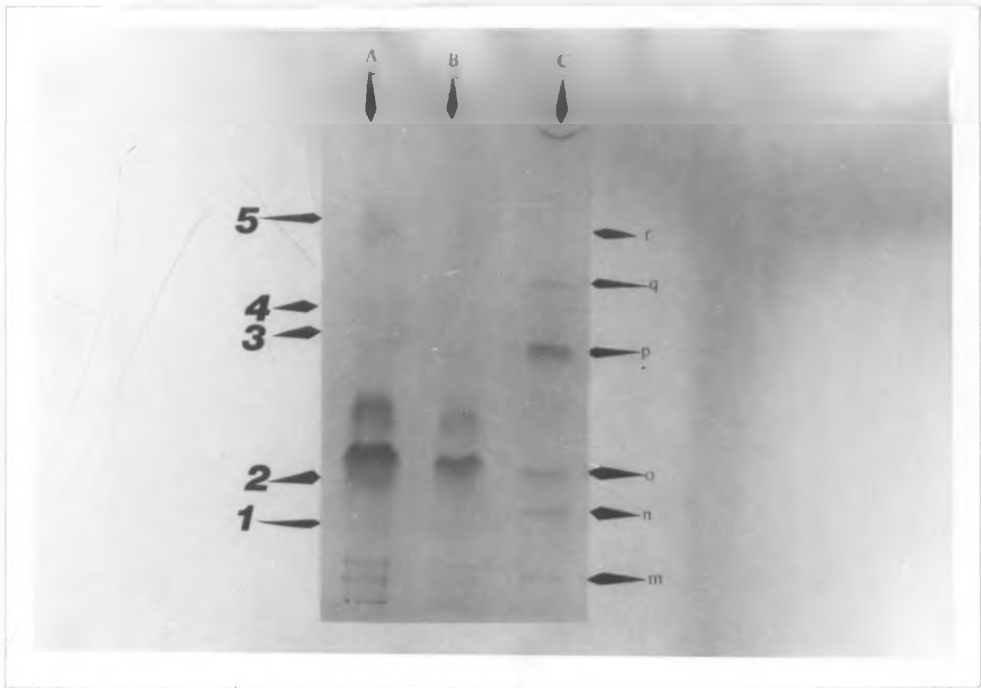
Note:

Molecular standards		Viral polypeptides	
m	97,400	1	71,753
n	68,000	2	62,426
o	43,000	3	32,581
p	29,000	4	25,120
q	18,400	5	6,466
r	14,300		

A Camelpox virus sample
B Control tissue culture fluid
C Molecular standards

133

standard molecular weight curve derived from the distance ration of the molecular weight markers after polyacrylamide electrophoresis
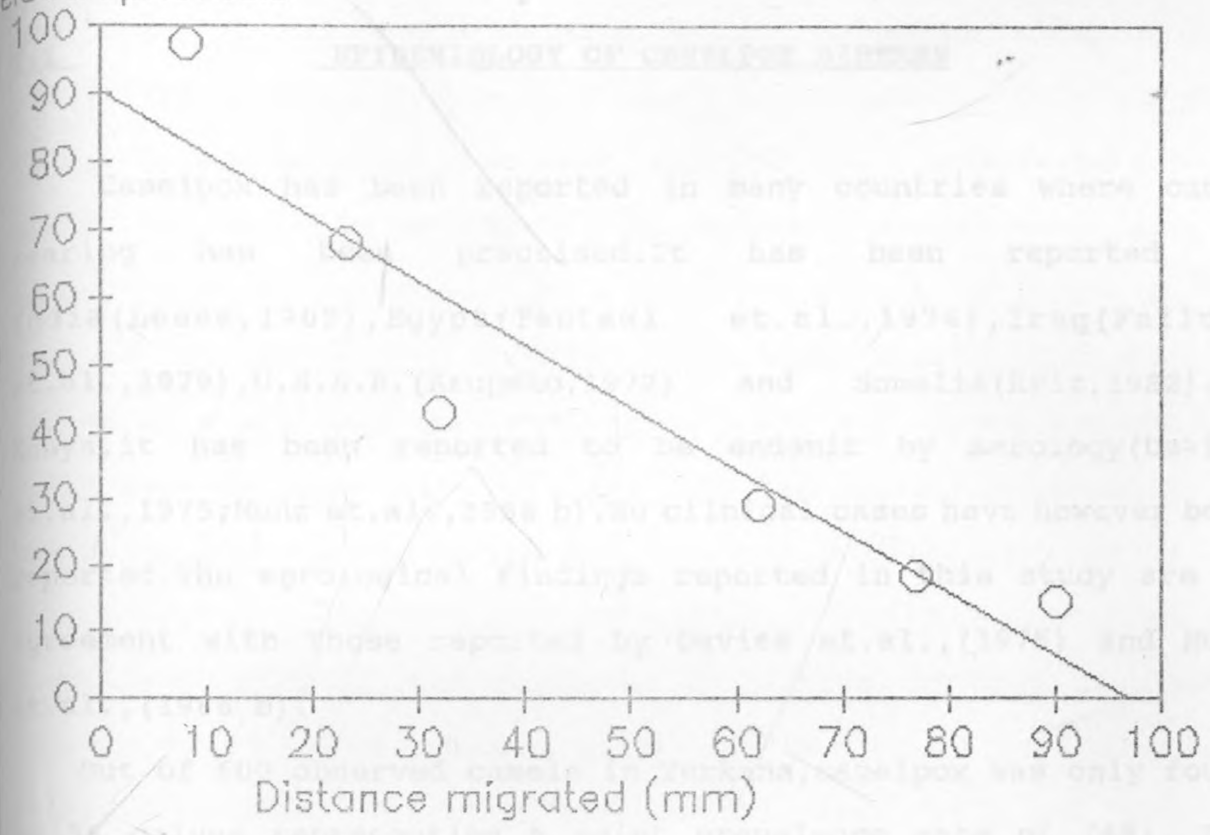
DISCUSSION

EPIDEMIOLOGY OF CAMELPOX DISEASE

Camelpox has been reported in many countries where camel rearing has been practised.It has been reported in India(Leese,1909),Egypt(Tantawi et.al.,1974),Iraq(Falluji et.al.,1979),U.S.S.R.(Krupeko,1972) and Somalia(Kriz,1982).In Kenya,it has been reported to be endemic by serology(Davies et.al.,1975;Munz et.al.,1986 b).No clinical cases have however been reported.The serological findings reported in this study are in agreement with those reported by Davies et.al.,(1975) and Munz et.al.,(1986 b).

Out of 600 observed camels in Turkana,camelpox was only found in 36 calves representing a point prevalence rate of (6%). The finding of only two outbreaks in Turkana would indicate a low incidence, however, that may be because our investigation was carried out in the dry season and that our investigations were not exhaustive. Most of the veterinarians and pastoralists interviewed indicated that most of the outbreaks are found in the wet season or immediately thereafter. One of the limiting factors that would hinder an investigation at such a time is the fact that these areas are impassable at such times. Other reports indicate that many outbreaks do occur in the rainy season and it is at such times that serious cases, some of them fatal, may occur(Mcgrane and Higgins, 1985). This is probably related to the fact that the calves are

135

weak and malnourished in the preceding dry period. The sudden onset of heavy rains in these areas puts the calves under severe stress due to the environmental changes. It is however interesting that two groups of camel calves in distant herds with different micro-climates were involved. One herd was in the very arid North area of Kibish, while the other was in the relatively moist south area of Lokori. Both areas however were dry at the time of investigation and had not experienced rain for about eight months. The affected calves were all less than one year old and had severe pustules on their muzzles, lips and nostrils. They had swollen cervical lymph nodes and had difficulty in eating. Such a clinical finding has been reported by McGrane Higgins (1985). The whole calf herd was normally involved in the outbreaks in Turkana, although no deaths were reported. This pattern of a 100% morbidity has also been reported by other workers(McGrane and Higgins, 1985) and has been explained by either the stress of weaning or poor nutritional status(McGrane and Higgins, 1985). Our findings indicate that the practice of night herding of all calves in the same "boma" in close contact could have also contributed considerably, since that would facilitate the quick spread of the virus via contact.

The clinical findings of camelpox in camels in Samburu,which were imported from Galana ranch and Somalia, were however more severe than those from Turkana. Adult camels of about five years were involved. This is in contrast with most workers who have found outbreaks in young camels and not in adults(McGrane and Higgins, 1985; Kriz, 1982). These camels had swollen heads and oedematous

136

faces, discharging eyes, swollen cervical lymph nodes, and were unable to feed properly for two weeks. There were no mortalities however. This finding suggests that camelpox can occur in any age group which is not immune. The most likely explanation for this outbreak in adult camels after moving from one area to another, would be that even though they may have been exposed previously to camelpox virus, they may have now come into contact with a more virulent strain or the immunity had waned. The fact that no other camels were involved in surrounding areas, would indicate that these local animals were immune to an endemic strain of camelpox virus. Unfortunately, there was no available sera from these animals prior to their sickness to test their immune status. It has been recognised before, that there are different strains of camelpox virus with different clinical diseases and differing morbidity and mortality(Kriz, 1982).

The problem of camel movement is at the moment minimal within the traditional set up, but as commercial camel ranching gets more popular in the exploitation of arid and semi-arid lands,and other events like the "Camel derby"(camel racing) recently introduced get underway, then more camels will move from one region to another. It will then be prudent that camels are vaccinated strategically before they move from one area to another just as it is done for many viral diseases of other animals like foot and mouth,sheeppox and lumpy skin disease in Kenya. Under the traditional set up, pastoralists in Kenya use camels mainly for milk and meat (Dahl, 1980). If they were to be used extensively for transport and

137

riding, then outbreaks of camelpox would be expected to be more frequent.

The camelpox lesions which were noticed in these investigation were however restricted to the head and neck region. Other investigators have reported generalized lesions throughout the body as in Somalia(Jazek *et.al.*, 1983) especially in calves. It is likely that some strains are more virulent than others.

In all the areas visited, there were no human infections by camelpox noticed or mentioned by pastoralists. It has been reported by unverified reports that people drinking milk could get infected with camelpox virus(Davies *et.al.*, 1975). In the investigation by Kriz (1982), only one person was suspected to be infected by a camelpox virus. It should be cautioned that camelpox virus is a potential risk in the post-smallpox eradication era (Baxby, 1988). It is important that an inactivated camelpox vaccine be used to control outbreaks of camelpox. It is, however, possible that the assumed camelpox infection in man is due to a parapox infection from camels and not camelpox virus.

5.2   CAMEL CONTAGIOUS ECTHYMA

Four outbreaks of camel contagious ecthyma (CCE) were found in camel calves in Turkana in four different areas with distinctly different eco-climatic settings. Two outbreaks were found in the arid north and two outbreaks in the south. The outbreaks were found in the dry season. The clinical signs of pustule formation on lips

and muzzles were very similar to those of camelpox infections. The two conditions are known to have similar clinical signs (Jazek et.al.,1983). The point prevalence of 10 % in Turkana district was high considering that no outbreak was found in Samburu district.

Our findings, however, were that in the four herds affected, goat kids were also affected. The goat kids were about four months old and were also suckling just like the camel calves. There were however no lesions on the teats of camel dams although there were small lesions on the teats of goat dams. Contagious Pustular dermatitis of other animals besides sheep and goats(Musk, Ox, reindeer, mountain goats and sheep; Stenbok, alpacas, dogs) is thought to be of ovine or caprine origin(Robinson and Balassu, 1981). Our findings suggest a similar pattern for CCE and contagious pustular dermatitis. In the previous report of CCE in camels in Kenya, (Munz et.al., 1986 a), sheep were also infected although not much significance was attributed to this fact. It may however be difficult to prove beyond doubt the actual origin of the virus.Other reports indicate that CCE is found in old and young camels(Munz et.al., 1986 a). The young camels of 1-4 years old have a more severe outbreak compared to adults, there is a marked generalization with lesions spreading to distal parts of the legs, inner parts of the thighs and, in the vaginal area in females. The morbidity was found to be about 100% in the young camels as compared to 10-20% in adult camels. In contrast, in this study, the lesions were only in calves less than one year old and lesions were not found in other parts of the body other than the muzzle,

139

lips and nostrils. The 100% morbidity involvement of young calves was similar to the finding by Munz *et.al.*, (1986 a).

One of the most interesting findings was the fact that the four outbreaks reported involved camel calves with the same stage of lesion development at the pustule stage, in camel herds from widely scattered locations in Turkana District. This would indicate the presence of some common predisposing element in the environment(Gitao, 1990).

While camelpox is caused by an orthopox virus,CCE is caused by a parapox virus and the two are distinct entities even though they have similar clinical signs and epidemiological patterns.Outbreaks of camelpox have been reported in herds which have had CCE(Munz *et.al.*,1986 a).Another interesting finding was the occurrence of streptothricosis in camel calves which was the first report of natural camel streptothricosis(Gitao *et.al.*,1990).This was in the same farm that camel contagious ecthyma had been reported(Munz *et.al.*,1986 a). It is quite probable that the two conditions could occur simultaneously in camels. Streptothricosis has been reported to occur simultaneously with contagious pustular dermatitis in sheep(Munz, 1969).

CAMELPOX   VIRUS PROPAGATION

5.3.1   EMBRYONATED EGGS

Inoculation of camelpox virus on the chorioallantoic membrane on which it produces dense white pocks, is said to be probably the optimum method for isolation and initial characterization of the virus(Fenner *et.al.*, 1989). This is in agreement with the study here where initial propagation was found most appropriate in embryonated eggs.

White pock lesions about 0.8-1mm.in diameter were produced six days post infection at 37^0 c and 39^0 c. At 34^0 C,slightly smaller pock lesions but with the same morphology were formed, the pock lesions were not elevated above the surface. This is in agreement with Davies *et.al.*, (1975) and Baxby,(1972). The highest temperature for pock production is said to be 38.5^0C(Baxby, 1972). Most of the pock lesions were fused at the point of inoculation, forming a mass of 3-4 cm. at the inoculation site. This mass was swollen and oedematous at the edges. Such fusion of camelpox pock lesions has been reported by Falluji *et.al.*,(1979); Tantawi *et.al.*, (1974); Ramyar and Hessami,(1972). Five pox virus strains from Egypt, Iran and the U.S.S.R.(Tantawi *et.al.*,1974) however, produced minute white pock lesions on infected CAM without generalization of the lesions.

In contrast,Marennikova *et.al.*,(1974) reported minute punctate pock lesions of 0.2 - 0.3 mm with central haemorrhage formed by camelpox virus.

5.3.2   HAEMAGGLUTINATION

The camelpox virus was found not to agglutinate chicken red blood cells. This is in agreement with Tantawi *et.al.*,(1974). Davies *et.al.*,(1975) found the H520 strain of camelpox virus to haemagglutinate poorly(1/8)as compared to vaccinia virus(1/64). Similar findings were reported for the etha strain of camelpox virus from Iraq (Falluji *et.al.*,1979).

5.3.3   TISSUE CULTURE

Clear cytopathogenic effects were produced in primary cells and secondary tissue culture cell lines. The primary tissue cultures which produced cytopathogenic effects were sheep kidney and sheep testis. These tissues have been used in the propagation of camelpox by other workers(Davies *et.al.*,1975; Ramyar and Hessami,1972). In this study, sheep kidney cells were found to be the most convenient means of propagation of camelpox virus. This was because of the availability and ease of obtaining lamb embryos and the easy and successful passage of kidney parenchymatous cells. The propagation of camelpox virus on sheep skin cells and sheep lung cells is reported for the first time. Sheep skin cells were however

difficult and tedious to propagate and passage.

The appearance of the infection centres was as reported by other workers. There was a focus of infected cells which eventually peeled off(Davies et.al.,1975; Ramyar and Hessami, 1972). The first signs of infection occurred two days after infection when various foci showed cell rounding, granulation and increase in refractoriness. Giant cells occurred four days after infection.These were large aggregations of 20 to 25 cells with inclusion bodies observed after staining just as reported by Tantawi et.al.,(1974). By the sixth day post inoculation, most of the cells were destroyed and the monolayers were peeling off. Other primary tissue cultures in which camelpox virus was propagated were the calf kidney and calf testis. Similar propagation has also been reported for camelpox virus(Tantawi et.al.,1974; Ramyar and Hessami,1972; Davies et.al., 1975). In this study, camelpox virus was also propagated on calf lungs and calf thyroid. The propagation pattern was similar to that on sheep primary cultures. Other primary tissue cultures on which camelpox virus has been propagated include chicken embryo fibroblasts(Falluji et.al.,1979), pig kidney (Ramyar and Hessami, 1972), camel embryo(Tantawi et.al.,1974)and rabbit kidney primary cells (Tantawi and Sokar, 1976). In this study, camelpox virus was propagated on Vero cells and Baby hamster kidney cells.Similar propagation has been reported by Davies et.al.,(1975).

143

PATHOGENICITY

Camelpox virus was not found to be pathogenic to many animals including rabbits, rats, mice, and chicken. This is in agreement with other workers, who found camelpox virus to have a narrow host range(Davies et.al.,1975; Baxby, 1972; Ramyar and Hessami, 1972). A mild transient erythema was produced in rabbits however, by the Etha strain of camelpox virus(Falluji et.al.,1979),while rabbits infected with an Egyptian strain of camelpox virus(Tantawi et.al.,1974) had nodular and ulcerative skin lesions. Monkeys were found to be susceptible to camelpox infection by developing pox lesions from which virus could be re-isolated(Falluji et.al.,1979). Camels have however been found to be susceptible to camelpox infection by most workers. The Egyptian strain of camelpox was found to produce pox lesions in camels ten days post infection(Tantawi et.al.,1974). The Tehran strain of camelpox virus(Ramyar and Hessami, 1972) caused generalized pox infections in young camels.Camelpox virus(Baxby et.al.,1975) was found to be very infective to camels even at a very low dose (1.8 X 10' TCID ./ml.). Virus could be recovered from primary as well as secondary lesions. In this study, camelpox virus caused infection in the camels which had not been vaccinated. In the test camels, there was a linear dose response curve indicating the replication of camelpox virus(Fig 27). This was most prominent on day 10 post challenge in both sheep skin and sheep kidney propagated camelpox viruses.

5.5 VIRUS INACTIVATION

5.5.1 FORMALIN INACTIVATION

In the work described here, camelpox virus was inactivated by formalin after 36hr and 48hr by 1:2000 and 1:4000 formaldehyde respectively. This is similar to the times described by Goyal and Singh(1975); Ramano Rao(1962) and McNeil(1965).

5.5.2 HYDROXYLAMINE HYDROCHLORIDE

Inactivation of vaccinia with hydroxylamine hydrochloride was found to be erratic and complete after a long exposure of 60-72 hr at $18-20^0$ C in the dark(Turner $et.al.$,1970). In this study, camelpox was inactivated after 24hr. and 36hr. respectively by 0.5M and 0.1M hydroxylamine respectively. This is in agreement with findings by Ramano Rao(1962), with vaccinia and Goyal and Singh(1975), with sheep pox virus.

5.5.3 ACETYLETHYLENEIMINE

Inactivation of camelpox with acetylethyleneimine was complete after 10 hrs in 0.05% Acetylethyleneimine. This is similar to the time obtained by Goyal and Singh(1975) on inactivation of sheeppox virus. Some of the dangers involved in using acetylethyleneimine include its being carcinogenic(Ramano Rao, 1962) and it was found

145

to produce a less immunogenic vaccinia virus vaccine after inactivation, as compared to formalin inactivation(Ramano Rao, 1962).

5.5.4 VACCINE IN RABBITS

The formalin inactivated vaccine was found to have no untoward effect on rabbits.There was a consistent antibody rise as depicted on Fig.17. Vaccinia another orthopox virus has shown similar antibody rise when inactivated and inoculated into rabbits (Boulter et.al.,1971).

5.6 CAMEL RESPONSE TO VACCINATION

5.6.1 DERMAL RESPONSE

The skin response of the vaccinated camels following challenge lasted for only five days. In contrast, some of the non vaccinated camels had pox lesions which went through typical pox stages of papule, vesicle, pustule and scab formation and that took upto twenty days. All the camels had lesions on the first three days of challenge due to hypersensitivity from the inoculated foreign material. The reaction subsided after three days in the control animals but continued in the test animals. This is similar to the results of Plowright et.al.,(1959) and Pyne(1990), when they were

146

using sheeppox vaccine. The fact that there was a linear dose response curve in the test animals would indicate the replication of camelpox virus. Many of the lesions on the test camels also had central necrosis which is common in many poxvirus infections(Plowright *et.al.*,1959).

The lack of difference between responses from sheep kidney and sheep skin propagated camelpox virus would indicate that the two viruses were identical in terms of pathogenicity.The theory that skin propagated camelpox virus would be more pathogenic as it has adapted on epidermal cells as compared to kidney propagated camelpox virus did not hold true.May be the theory could hold true if many passages (>50) were to be performed but this is not easy as skin cells are very difficult to propagate.

The antibody profile showed an increase in titre in the vaccinated camels upto day 21. The unvaccinated camels had no antibodies to camelpox until the day of challenge. After challenge, the antibody titre rose rapidly probably due to the active infection unlike in the control animals. By day 31 post vaccination(day 10 post-challenge), however, the vaccinated camels had a significantly higher titer than the unvaccinated camels(p<0.05). There was therefore a correlation between protection as determined by the skin response and the serological response. The titers in the unvaccinated camels rose steadily probably due to the active infection .

In describing similar work, McNeil(1965)found that inactivated vaccinia virus can produce an increase in skin resistance to live

virus in rabbits and that this resistance is dependent on the quantity of antigen in the vaccine. Furthermore, the degree of skin resistance is related to the level of circulating neutralizing antibody and not to the degree of delayed hypersensitivity. These reports are in agreement with the findings reported here and those by Parker and Rivers, (1936) and Collier *et.al.*,(1955) but in conflict with results reported by Andrewes *et.al.*,(1948);Amies, (1961) and Ramano Rao, (1962) who reported that inactivated vaccinia virus vaccines do not produce an increase in skin immunity.

Other investigators consider that inactivated vaccinia virus can induce skin immunity but that there is no relationship between this and the level of circulating antibody in man, (Kaplan, 1960; Beunders *et.al.*,1960), and in rabbits(Turner *et.al.*,1970). Nevertheless, rabbits immunized with killed vaccinia virus, while not protected from infection survived challenge with lethal doses of rabbit pox(Boulter *et.al.*,1971; Madeley, 1968). Mice were also protected in a similar way with killed vaccines(Turner *et.al.*,1970).

In explaining the results,McNeil(1965) stated that regular production of skin immunity was dependent on the use of vaccines containing high concentrations of virus(10^8 pfu/dose)before inactivation. Secondly, the relationship between skin resistance and antibody titre was not a precise one and was only likely to be apparent when large numbers of animals(97 rabbits in his studies) were used. Thirdly, the method of measuring skin immunity was

important and if qualitative, may not be as revealing as semi-quantitative(McNeil, 1965).

Other workers have been able to reproduce generalized skin lesions after inoculation of camelpox in camels(Baxby *et.al,.*1975). This is probably related to the virulence of the particular strain of camelpox virus or susceptibility of test animals. In the non-vaccinated camels inoculated in the experiment reported here, there was no generalization of the skin lesions. This is not surprising, considering that in the epidemiological investigations of natural cases, generalized skin lesions of camelpox infection were not found. It may be that the strains of camelpox virus found here in Kenya are of less virulence than in other areas or that Kenyan camels are less susceptible. Nevertheless, it is unlikely that vaccine developed with this relatively non virulent strain may prevent infection and generalization of lesions after infection with the virulent strain, whether a live or inactivated vaccine was used.

Other attempts at control of camelpox have involved "variolation" as described by Leese(1909). In the Punjab and Rajputana,camel breeders inoculated young camels with virulent material before the onset of rains, thus assuring a mild attack and lifelong immunity. Such a method may lead to uncontrolled spread of the virus not only in susceptible camels, but to other animals for example rhesus monkeys which are susceptible by experimental infection(Baxby, 1972, Falluji *et al.*,1979). The use of an inactivated vaccine as described in this study avoids these problems since the virus is

inactivated.

The use of vaccinia virus to vaccinate susceptible camels has been suggested by some workers(Fenner *et.al.*,1989) but this is qualified with the statement that care has to be taken in choosing a strain of vaccinia virus that does not cause a generalized disease in young camels and spread from one camel to another, or possibly man. Vaccinia virus has been reported to cause camelpox disease in camels(Krupenko,1972). The use of an inactivated vaccine as described in this study avoids this danger since the camelpox virus is not replicating.

The other alternative vaccine would be a live camelpox vaccine which has been adequately passaged. Such a live vaccine would give a more potent immunogenic response and for a longer period than an inactivated vaccine. It would also have some disadvantages in that if it were to revert to virulence in camels, it would pose a serious danger to camels.

5.6.2   ABSCESS DEVELOPMENT

Some of the sites of challenge on the camels developed abscesses.This was mainly due to infection of the sites with skin contaminants. The fact that haemolytic pathogenic *staphylococcus aureus* was isolated indicates that some of the skin contaminants are pathogenic. Many of the natural infections are most likely to be invaded by *S. aureus* as a secondary invader. Secondary

infections are the most likely causes of death in natural infections(Pandey *et.al.*,1985).

5.7 CAMELPOX VIRUS POLYPEPTIDES.

Only four distinct polypeptides were visible after electrophoresis.This was a small number compared to other workers who have been able to identify about 80 polypeptides(Pennington, 1974)and 279 polypeptides(Carrasco and Bravo, 1986)for vaccinia virus. The low number is probably due to the low resolution of the electrophoresis method used.

CONCLUSION

In this study,camelpox was found to be present in Turkana and Samburu,two main camel rearing areas in Kenya.While camelpox was found in camel calves in two outbreaks from Turkana,it was also found in adults in two outbreaks in Samburu.This indicates that camelpox may be found in any age group depending on the immune status.While in calves the main lesions were pustules on the muzzle and mouth regions,in adults there was also in addition severe neck swelling on the mandible region.Cervical and mandibular Lymph node enlargement was also present.

Camel contagious ecthyma a closely related pox disease was found in four outbreaks in Turkana involving only camel calves and in all cases,goat kids were also affected. In all cases, the camels were severely affected having pustules, lymph node enlargement and were unable to feed. They also had secondary bacterial infection.

Several tissue cultures were used to propagate camelpox and sheep kidney cells were found to be the most suitable and convenient. Characteristic giant cells(syncytia) were regularly formed by the virus in these cells. Camelpox virus was found to be non-pathogenic to many laboratory animals. Five polypeptides were identified after polyacrylamide gel electrophoresis of the virus. Of three inactivating reagents, formalin was found to be the most suitable in inactivating camelpox virus and this was done on bulk camelpox virus. This virus was combined with an adjuvant and used on rabbits where it was found to have no side effects. This vaccine

152

was then used on twenty camels which were then challenged twenty one days post vaccination. A group of ten camels which had not been vaccinated were also challenged. The vaccinated camels had been protected as their skin response to challenge was mild and healed in five days while the non vaccinated camels developed lesions which were more severe and took longer to heal. The vaccinated camels also had significantly higher antibody titres than the non vaccinated camels. The vaccine was therefore useful and could be used to protect camels before movement to camelpox endemic areas. There would be no risk of spread involved since the vaccine is inactivated.

6.1 RECOMMENDATIONS

1. Camelpox is an enzootic disease in camel rearing areas of Kenya affecting both young and mature camels which are not immune.It is recommended that before weaning, camels should be vaccinated to reduce the incidence of camelpox. This is even more so if the camels are to be moved from one area to another.

2. It is recommended that the inactivated vaccine against camelpox be used to protect camels before weaning as described here but since the protection was studied for one month,a booster may be necessary after six months.

3. Camel contagious ecthyma is a disease that closely resembles camelpox but affects mostly young camels before one year and tends to be found in areas with caprine or ovine parapox infections.

153

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APPENDICES

Concentration of camelpox virus using hollow fibre Electronic lab assistance software.

Variables used	Control	Test
Initial volume	500 ml	1000 ml
Macromolecular concentration	1 mg/ml	1 mg/ml
Minimum acceptance % recovery	93	93
Final volume	50ml	100ml
Operating temperature	$4\ ^0$ C	$4\ ^0$ C
Initial vacuum	0.1mm HG	0.1mmHG
Operation mode	Single	Single
HFCF* available for use	Orange	Orange

HFCF* = Hollow fibre concentration filter

Required set for optimal concentration of test and control fluid

Variables used	Test(virus)	control
Optimum HFCF* configuration	Single	Single
Length of run	10hr 30 min	5hr 15min
Sample head	58 cm	59 cm
Dialysis volume	100ml/hr	100 ml/hr
Rate of concentration/filtration	10 X	10 X
%Recovery	93 %	93 %
Drip	Orange	Orange
Temp	4 ^{0} C	4 ^{0} C

HFCF* = Hollow fiber concentration filter

Gel preparation

Materials and Methods used in Polyacrylamida gel electrophoresis preparation

Polyacrylamide preparation.

Acrylamide-bisacrylamide was prepared by dissolving 30 g of acrylamide and 0.8 g of bisacrylamide in a total volume of 100 ml of water.The solution was filtered through Whatman No. 1 filter paper,and stored at 4 ^{0} in a dark bottle.Temed was used as supplied.It was stored at 4 ^{0} in a dark bottle.Ammonium persulfate (1.5 %,w/v), was prepared by dissolving 0.15g Ammonium persulphate in 10 ml of water.A fresh preparation was made when need arose.SDS solution(10%,w/v) was prepared by adding 10 g of Sodium dodecyl sulphate(SDS) in water to make 100 ml

Composition of polyacrylamide gel (10%)

Formula for 10% gel preparation (SDS-Discontinuous buffer

Item used	stacking gel (ml)	Acrylamide conc Resolving gel in (a)(ml)	Reservoir buffer (ml)
Acrylamide-bisacrylamide	2.5	10	
stacking gel buffer stock	5.0		
Reservoir buffer stock			100
Resolving gel buffer stock		3.75	
10 % SDS	0.2	0.3	
1.5 % Ammonium persulfate	1.0	1.5	
Water	11.3	14.45	90
TEMED	0.015	0.015	

The buffers used were prepared as described below:

A Final concentration of buffers:

   Stacking gel;0.125 M Tris-Hcl,pH 6.8

   Resolving gel;0.375 M Tris-Hcl,pH8.8

   Resolving buffer;0.025 M Tris,0.192 M glycine,pH 8.3

a) The columns represent volumes(ml)of the various reagents required to make 30 ml of gel mixture

b) Volumes(ml) of reagents required to make 1 litre of reservoir buffer

B) Stacking gel buffer stock:0.5M Tris-Hcl(pH6.8);6.0g of Tris is dissolved in 40 ml of water,titrated to pH 6.8 with 1 M Hcl(48 ml)and brought to 100 ml final volume with water.The solution is filtered through whatman No 1 filter paper and stored at 4 ^{0} C.This

gives   0.125 M Tris-Hcl pH 6.8

C) Resolving gel buffer stock:3.0 M Tris-Hcl(pH 8.8);36.3 g of Tris and 48.0 ml of 1M Hcl are mixed and brought to 100 ml final volume with water.This buffer is then filtered through Whatman No.1 filter paper and stored at 4 ^{0} C.This gives a 0.375 M Tris-Hcl buffer,pH 8.8

D)   Reservoir   buffer   stock:0.25   M   Tris,1.92   M   glycine,1 %SDS(pH8.3);30.3 g of Tris,144.0 g of glycine,and 10.0g of SDS are dissolved in and made to 1 litre with water.The solution is stored at 4 ^{0} C. This gives a 0.025 M Tris, 0.192 M glycine, pH8.3

APPENDIX V

Sample preparation

A: Sample preparation

	Sample volume (ul)	sample buffer vol.(ul)	water (ul)
Virus sample	50	25	
Control	50	25	
Markers	10	25	4 0

B: Sample buffer   0.0625 M Tris-Hcl(pH 6.8)

2% SDS

5% 2-Mercaptoethanol

10% Sucrose

0.002% Bromophenol Blue

APPENDIX VI

Gel staining

A: Staining Solution:

Coomassie Blue R-250(0.1%) 20mg. dissolved in water:methanol:glacial acetic acid (5:5:2 by vol)and filtered through a Whatman No.1 filter paper to make 1 litre of solution

B: Destaining solution:

12.5 % isopropanol,10% acetic acid in Tris-Hcl buffer(pH6.8)

APPENDIX VII

Molecular markers

The molecular weights of standard markers

Protein	Molecular Weight
Lysozyme	14 300
B Lactoglobulin	18 400
Carbonic anhydrase	29 000
Ovalbumin	43 000
Bovine serum albumin	68 000
Phosphorylase B	97 400
Myosin(H Chain)	200 000

The standards from Bethesda Research Laboratories life Technologies,Inc. are supplied in lyophilized form and upon reconstitution with 500 ul of 1 millimolar yield a solution of 10

mm   Tris-HCL(pH   7.2),2   %(w/v)SDS,and   1   mmDTT(Millimolar Dithiothreital).

After reconstitution,each protein is present at a concentration of approximately 1 mg/ml