The characterization of African strains of capripoxvirus

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SUMMARY

Isolates of capripoxvirus collected from sub-Saharan Africa were compared in sheep, goats and cattle and by restriction endonuclease digestion of their purified DNA. Biochemical techniques were used to precisely identify strains of capripoxvirus for epidemiological investigations. Strains of capripoxvirus infecting cattle have remained very stable over a 30-year period and are closely related to strains recovered from sheep in Africa.

INTRODUCTION

Epidemiological investigation of endemic virus diseases relies heavily on the ability to distinguish between closely related strains of virus. However, until recently it has not been possible to distinguish between strains of capripoxvirus using standard laboratory tests, even when the behaviour of these strains in the field situation has clearly been different (Kitching & Taylor, 1985). The capripoxviruses cause sheep pox, goat pox and lumpy skin disease (Neethling) of cattle and are economically the most important group of poxviruses. Most strains of capripoxvirus do not have absolute host specificity (Capstick, 1959; Kitching & Taylor, 1985) and while the majority of those examined showed a preference for growth in either sheep, goats or cattle, some strains had no obvious host preference. Strains also varied in their pathogenicity in different species and in different breeds (Capstick, 1959; Kitching & Taylor, 1985).

Lumpy skin disease (Neethling) was first diagnosed in Kenya in 1957 (Burdin & Prydie, 1959), and was associated with an outbreak of sheep pox in indigenous fat tailed sheep on the same farm. This association was at first thought significant, but as the outbreak of lumpy skin disease (LSD) progressed it became clear that sheep were neither responsible for spreading the disease between farms, nor clinically susceptible when in contact with infected cattle (MacOwan, 1959). However, Capstick & Coackley (1961) showed that cattle could be immunized against LSD using Kenyan strains of sheep pox virus designated by them Kedong, Isiolo and SP143.

Black, Hammond & Kitching (1986) have compared the DNA fragments generated by *Hind*III restriction endonuclease digestion of 12 field and vaccine strains of capripoxvirus and were able to distinguish between them. Subsequent studies on seven of these strains (Gershon & Black, 1988) emphasized the high degree of nucleotide sequence homology, shared by all strains of capripoxvirus.

Table 1. *Strains of capripoxvirus, origin and year of infection*

Strain	Origin	Species	Year of isolation	Reference
2490	Kenya	Bovine	1958	Davies, 1982
1581 (Gough)	Kenya	Bovine	1959	Davies, 1982
3906 (Starnes)	Kenya	Bovine	1959	Davies, 1982
Londiani	Kenya	Bovine	1959	Capstick, 1959
Isiolo	Kenya	Ovine	1959	Capstick, 1959
Kedong	Kenya	Ovine	1955	Capstick & Coackley, 1961
SP143	Kenya	Ovine	Not recorded	Capstick & Coackley, 1961
0240/KSGPV (field)	Kenya	Ovine	1974 ?	Davies, 1976
SA vaccine	S. Africa	Bovine	Not recorded	Kitching, Hammond & Black, 1986
SA virulent LSD (Neethling)	S. Africa	Bovine	1977	
Kenya vaccine	Kenya	Ovine	1974	Davies & Mbugwa, 1985
Kenya LSD B507	Kenya	Bovine	1987	
Nigeria LSD	Nigeria	Bovine	1974	Nawathe <i>et al.</i> 1978
Cameroon LSD	Cameroon	Bovine	1986	
0240/KSGP (vaccine)	Kenya	Ovine	1974 ?	Kitching, Hammond & Taylor, 1987

Certain of these genome characteristics were used by Black, Hammond & Kitching (1986) and Gershon & Black (1988) to place the strains into groups which correlated with the species, cattle, sheep or goat, from which each had been originally isolated, although there was evidence to suggest that two of the strains were recombinant viruses, sharing characteristics of two of these groups.

This paper describes the use of experimental animals and digestion of the virus DNA with restriction endonucleases to specifically characterize a range of African isolates of capripoxvirus, in an attempt to explain some of the conflicting epidemiological field and laboratory observations.

MATERIALS AND METHODS

Viruses. The strains of capripoxvirus used in the present study are listed in Table 1.

The initial passage history of these strains had not been recorded, although all had been passaged at least once on lamb testis (LT) cells. The 0240/KSGPV, B507 and Kenya LSD vaccine strains had been supplied by Dr G. Davies, as freeze dried preparations, originally isolated and passaged on LT cells or bovine foetal muscle cells. The South African vaccine strain of LSD and virulent (Neethling) strain of LSD were supplied by Dr B. Erasmus of the Veterinary Research Institute, Onderstepoort, Republic of South Africa. The isolate of LSD virus from Cameroon was sent by Dr Maikano Abdoulaye of the Ministry of Livestock, Fisheries and Animal Industries, Garoua, Republic of Cameroon.

The freeze dried viruses were reconstituted with sterile distilled water and passaged in secondary cultures of LT cells as previously described (Kitching & Taylor, 1985). The virus strains used to inoculate sheep, goats and cattle were

passaged once more onto secondary LT cells, and the cultures frozen to -20°C when 90% of the cells were showing a cytopathic effect (cpe) due to virus growth. These were then thawed, the harvest centrifuged at 1000 g for 10 min, and the supernatant titrated and used as the inoculum. Aliquots of virus harvest from the first LT passage of each of the strains, including those not used for animal inoculation, were passaged three more times on LT cells. Nine of the strains were then purified, the viral DNA extracted and digested using *Hind*III, *Bam* HI or *Pst* I restriction endonuclease, and the resulting DNA fragments separated on agarose as previously described (Black, Hammond & Kitching, 1986).

Animals. Fifty-five yearling cattle, 30 yearling crossbred goats and 37 yearling crossbred sheep were used in the experiments and housed in groups in the high security isolation facilities at IAH, Pirbright. Sheep and goats were each inoculated intradermally with 0.2 ml of tissue culture supernatant, containing between $\log_{10}$ 3.6 and $\log_{10}$ 4.6 TCID₅₀ of capripoxvirus into a shaved area on the left flank over the last rib. Cattle were inoculated subcutaneously with between 2 and 3 ml of tissue culture supernatant containing between $\log_{10}$ 4.6 and $\log_{10}$ 5.8 TCID₅₀ of capripoxvirus into a clipped area over the left shoulder. Preinoculation and 21 day post-inoculation serum samples were collected from each animal, and these were stored at -20°C ; each animal was clinically examined daily and rectal temperatures recorded.

RESULTS

The clinical reactions following inoculation are summarized in Table 2. The strains 0240/KSGPV field, 3906, 0240/KSGP vaccine, SA (Neethling) LSD, Kenya B507 and Cameroon LSD produced only a single granulomatous reaction in both sheep and goats at the site of inoculation, and none of the animals developed a pyrexia greater than 40°C .

The three sheep inoculated with strain SP143 developed 3–4 cm diameter inoculation site lesions and a pyrexia greater than 40°C on day 6 after inoculation (p.i.) followed by the appearance of multiple secondary papules over the body; the two goats developed a pyrexia on day 7 p.i., 3 cm diameter inoculation site lesions, followed by the appearance of less than 10 secondary papules. The prescapular lymph nodes on all five animals were grossly enlarged.

The sheep and goats inoculated with the Kedong and Isiolo isolates developed severe capripox. The inoculation site lesions were between 4 and 7 cm diameter, poorly separated at their circumference from surrounding skin and centrally cyanotic. The animals had all developed a pyrexia by day 7 p.i., multiple secondary papules over the body, grossly enlarged superficial lymph nodes, rhinitis and conjunctivitis. The three sheep inoculated with the Isiolo strain died between days 12 and 13 p.i., and one of the sheep inoculated with the Kedong strain died on day 13 p.i.

On post-mortem examination only the sheep and goats inoculated with the Kedong and Isiolo strains had gross internal lesions, with papules in the lungs, abomasal mucosa and in the wall of the rumen.

Eight of the strains being investigated were inoculated into groups of cattle. Nine out of 10 cattle inoculated with the Kedong and Isiolo (sheep derived) strains

Table 2. *Clinical reactions in cattle, sheep and goats inoculated with strains of capripoxvirus*

Strain	Reaction at injection site			Enlarged regional lymph node			All superficial lymph nodes enlarged			Pyrexia			Secondary papules			Death		
	C Sh G			C Sh G			C Sh G			C ¹ Sh ² G ²			C Sh G			C Sh G		
	C	Sh	G	C	Sh	G	C	Sh	G	C ¹	Sh ²	G ²	C	Sh	G	C	Sh	G
3906	N.D.	3/3	2/2	N.D.	0/3	0/2	N.D.	0/3	0/2	N.D.	0/3	0/2	N.D.	0/3	0/2	N.D.	0/3	0/2
SP143	N.D.	3/3	2/2	N.D.	3/3	2/2	N.D.	3/3	2/2	N.D.	3/3	2/2	N.D.	3/3	2/2	N.D.	0/3	0/2
Kedong	4/5	3/3	2/2	4/5	3/3	2/2	1/5	3/3	2/2	1/5	3/3	2/2	0/5	3/3	2/2	0/5	1/3	2/2
Isiolo	5/5	3/3	2/2	0/5	3/3	2/2	0/5	3/3	2/2	0/5	3/3	2/2	0/5	3/3	2/2	0/5	3/3	2/2
S. Africa LSD vaccine	3/5	N.D.	N.D.	3/5	N.D.	N.D.	0/5	N.D.	N.D.	0/5	N.D.	N.D.	0/5	N.D.	N.D.	0/5	N.D.	N.D.
S. Africa virulent LSD	3/4	3/4	N.D.	2/4	2/4	N.D.	2/4	0/4	N.D.	2/4	0/4	N.D.	1/4	0/4	N.D.	0/4	0/4	N.D.
KSGP 0240 field	N.D.	3/3	2/2	N.D.	0/3	0/2	N.D.	0/3	0/2	N.D.	0/3	0/2	N.D.	0/3	0/2	N.D.	0/3	0/2
KSGP 0240 vaccine	9/13	15/15	15/15	6/13	0/15	0/15	3/13	0/15	0/15	3/13	0/15	0/15	3/13	0/15	0/15	0/13	0/15	0/15
Kenya LSD vaccine	3/5	N.D.	N.D.	3/5	N.D.	N.D.	2/5	N.D.	N.D.	2/5	N.D.	N.D.	0/5	N.D.	N.D.	0/5	N.D.	N.D.
Kenya LSD B507	4/5	N.D.	1/2	3/5	N.D.	0/2	3/5	N.D.	0/2	3/5	N.D.	0/2	2/5	N.D.	0/2	1/5	N.D.	0/2
Nigeria LSD	3/3	N.D.	N.D.	2/3	N.D.	N.D.	2/3	N.D.	N.D.	2/3	N.D.	N.D.	2/3	N.D.	N.D.	0/3	N.D.	N.D.
Cameroon LSD	N.D.	2/3	3/3	N.D.	1/3	1/3	N.D.	0/3	1/3	N.D.	0/3	0/3	N.D.	0/3	0/3	N.D.	0/3	0/3
Total	33/45	35/37	29/30	22/45	12/37	7/30	13/45	9/37	7/30	13/45	9/37	6/30	8/45	9/37	6/30	1/45	4/37	4/30

N.D., Not done; 1, Rectal temperature > 39.5 °C; 2, Rectal temperature > 40.0 °C; C, Cattle; Sh, Sheep; G, Goats.

Table 3. *Clinical reaction in cattle inoculated with serial passages of KSGP 0240 vaccine*

Source of infection	Animal no.	Biopsy	Maximum diam. of primary reaction (cm)	Enlarged regional lymph node	All superficial lymph nodes enlarged	Maximum pyrexia (°C)	Secondary papules
KSGP vaccine	PU 13	PU 13	5	+	—	40·0	—
	PU 14		2	—	—	39·8	—
	PU 15		1	—	—	38·5	—
PU 13	PP 87		2	+	—	N.D.	—
	PP 88	PP 88	6	+	—	N.D.	—
	PP 89		0·5	—	—	N.D.	—
PP 88	PW 70		12	+	—	38·7	—
	PW 71	PW 71	12	+	—	39·4	+
PW 71	RA 74		2	—	—	38·6	—
	RA 75		20	+	—	39·5	—

Table 4. *Relative sizes of HindIII fragments of capripoxvirus isolates*

Mol. wt. (kb)	Isolates			
	2490 Londiani KSGP 0240 vaccine Cameroon LSD	1581 3906	SP143 Kedong	Isiolo
18.00	—	—	—	A
12.00	—	—	A	—
11.4	A	A	—	—
10.7	—	—	B	B
8.6	BB*	BB	C	C
7.4	C	C	D	D
7.0	DD	DD	EE	EE
6.6	EE	EE	FFF*	FFF
6.2	—	—	GG	GG
5.5	F	F	—	—
5.4	G	G	H	H
5.25	H	H	—	—
5.1	II	II	I	I
5.0	—	—	J	J
4.8	—	—	K	—
4.4	J	J	L	K
4.35	K	K	M	—
4.25	—	—	—	L
4.0	L	L	N	M
3.8	M	M	O	N
3.6	N	N	P	—
3.47	O	O	Q	O
3.0	PP	PP	RR	PP
2.75	QQ	QQ	—	—
2.56	R	R	—	—
2.51	—	—	S	—
2.4	S	S	T	Q
2.3	TT	TT	U	R
1.91	—	—	V	SS
1.82	U	U	W	T
1.26	V	V	—	—
1.13	WW	—	—	—
1.1	X	W	X	U
1.0	Y	XX	YY	V
0.68	Z	Y	Z	W
0.66	1A	Z	1A	X
0.54	1B	1A	—	—
0.53	1C	1B	1B	Y
0.42	1D 1D	1C 1C	1C 1C	ZZ
0.32	1E	1D	1D	1A
0.24	1F	1E	1E	1B
No. fragments	41	39	38	35

* Double and triple letters indicate co-migrating fragments.

developed a local swelling of between 2 and 10 cm at the site of inoculation, and one of these developed a pyrexia of 39.6 °C on day 7 p.i. The South Africa LSD vaccine strain produced swellings at the inoculation site of between 5 and 10 cm on 3 of 5 cattle inoculated, but no evidence of a generalized reaction in any of the 5 animals. However, 9 of the 17 cattle inoculated with South Africa virulent, the Kenya LSD vaccine, the Kenya LSD B507 and the Nigeria LSD strains (cattle derived) developed a pyrexia and one of these animals died. The 0240/KSGP vaccine strain, although derived from a sheep, caused a more severe reaction in cattle than the Kedong and Isiolo sheep derived strains, producing a mild generalized reaction in 3 of the 13 cattle inoculated.

The reaction produced in cattle by the 0240/KSGP vaccine strain was further investigated by serially passaging biopsy material collected from individual animals which developed large local reactions following inoculation. An inoculum containing $\log_{10} 4.7$ TCID₅₀ was inoculated into three cattle (Table 3), one of which developed a large local reaction. Biopsy material from this animal was homogenized and a suspension containing $\log_{10} 5.3$ TCID₅₀ inoculated into another three cattle. In this manner the virus was passaged through two further groups of two cattle. One of these cattle, on passage three, developed a small number of secondary papules on the neck and shoulder. There was no indication that serial passage of the 0240/KSGP increased its virulence in cattle, or selected out a more virulent strain of virus.

The *Hind*III restriction digest patterns of the nine isolates were very similar (Table 4). Using the restriction endonuclease *Hind*III the Londiani, 0240/KSGP vaccine, 2490 and Cameroon strains were identical, the 3906 and 1581 strains appeared identical, and the Kedong and SP143 strains were also indistinguishable. The Isiolo strain differed from the Kedong and SP143 strains in seven fragments and the Londiani group differed from the 3906 and 1581 strains by the presence of the double w fragments. However, 18 differences could be distinguished between the cattle (3906) and sheep (SP143) strains. The strains can therefore be placed into two major groups which, apart from the 0240/KSGP, correlate with the animal from which each was derived. This grouping was confirmed using the restriction endonucleases *Bam* HI and *Pst* I (results not shown).

DISCUSSION

Lumpy skin disease (Neethling) was first diagnosed in cattle in Zambia in 1929 (see review by Weiss, 1968) and had spread into South Africa by 1944 where it has remained enzootic. Unlike the situation in South Africa in which eight million cattle were affected with up to 75% mortality on some infected premises, the disease in Kenya in 1958-9 was relatively mild with only 10 LSD associated deaths, and was quickly brought under control (MacOwan, 1959). A characteristic of the epidemiology of LSD is the appearance of the disease in epizootics which appear to spread north from Southern Africa. In 1970 the disease spread north into Sudan and then west into Nigeria in 1974, and Mauritania and Liberia in 1977. In 1981 a new epizootic of LSD was reported, which between 1981 and 1986 had affected Tanzania, Kenya, Zimbabwe, Somalia and the Cameroons, with mortality rates up to 20%. However, the true extent of the epizootic was not clear, and probably also affected a considerable area of central Africa.

The appearance of LSD has only once, in Kenya, been associated with capripox in sheep or goats, and on that occasion its subsequent spread was not related to the movement of sheep. In South Africa capripox has never been reported in sheep or goats. However, the extremely close antigenic (Capstick, 1959) and biochemical (Kitching, Hammond & Black, 1986; Black, Hammond & Kitching, 1986) relationship between the viruses which cause LSD, sheep pox and goat pox, together with the observations that these viruses can infect and cause disease in more than one species of host (Kitching & Taylor, 1985) and may recombine in the field (Gershon *et al.* 1988) indicate that they are all strains of a single species of virus. The enigma of LSD is its relatively recent origin compared with sheep pox and goat pox and why it has been restricted in its distribution to Sub-Saharan Africa. Gershon & Black (1988) have suggested that cattle strains of capripoxvirus are more closely related to sheep strains than goat strains, while Davies (1982) reported evidence that strains of capripoxvirus causing LSD are maintained in buffalo, and that epizootics of LSD occur when the virus spreads from buffalo into a sufficiently large susceptible cattle population. More recently Isitor *et al.* (1988) have shown evidence that cattle can be chronically affected with capripoxvirus, associated with dermatophilus infection. However, this form of maintenance of the virus would predispose to its movement on export cattle out of Africa, and it should be noted that LSD has never been confirmed outside Africa.

The sheep and cattle derived Kenya strains of capripoxvirus were shown by Capstick (1959) to be sufficiently closely related to cross immunize and experimentally infect sheep, goats and cattle. Ninety-seven of the 112 animals inoculated with the strains in the present study developed a clinically identifiable reaction at the inoculation site. All the inoculated animals seroconverted to capripoxvirus, and both the goats inoculated with the Kenya LSD B507 were subsequently resistant to challenge with the virulent Yemen capripoxvirus strain, even though one of these animals did not clinically respond to the B507 LSD inoculation.

The extremely variable clinical response of cattle to natural infection with capripoxvirus has been described by Weiss (1968) and Woods (1988). Similarly, the clinical response of the cattle in this study was unpredictable, and varied considerably between cattle of similar breed and age. The five cattle inoculated with the Kenya LSD vaccine were all 10-month-old Friesian cross Hereford heifers. Three of these animals were from one farm; these developed a 6–15 cm diameter swelling at the inoculation site, and two developed a pyrexia. The two remaining animals failed to react clinically to the inoculation of the vaccine strain, although both seroconverted to capripoxvirus. No histopathological study was made of the reaction in any of the experimental cattle, but the gross characteristics of the lesion at the inoculation site had much in common with that described by Roitt, Brostoff & Male (1985) as a granulomatous hypersensitivity reaction. We speculate that the reaction to inoculation of cattle with capripoxvirus may be associated with previous contact with parapoxvirus infection such as bovine papular stomatitis or pseudocowpox; Kitching, Hammond & Black (1986) have shown that the parapoxviruses share a major antigen with the capripoxvirus.

Restriction endonuclease digest patterns have provided very strong molecular evidence for the comparative grouping of different isolates of capripoxviruses

(Black, Hammond & Kitching, 1986, Gershon & Black 1988). The restriction endonuclease *Hind*III has proved to be the most useful in this respect as it produces approximately 40 fragments with a wide range of sizes. A *Hind*III digest pattern thus provides a rapid and very characteristic identification of the genome of a capripoxvirus isolate.

The restriction endonuclease digest patterns obtained in the present work show that the genomes of the cattle isolates of capripoxvirus, collected over a period of 30 years, demonstrate a remarkable degree of stability. Of the seven isolates studied, five were identical and the other two differed only by the presence of two additional small fragments (1·13 kb). This genomic stability is even more striking when the *Hind*III restriction digest pattern of the genome of the virulent Londiani isolate is compared with that of the highly adapted egg passage South Africa LSD vaccine strain. Between these two widely different viruses there are only three fragments different (Black, Hammond & Kitching, 1986; Gershon & Black, 1988).

Within the four sheep isolates the SP143, Kedong and the Isiolo isolates form a very close group. However, the fourth sheep isolate 0240/KSGP appears to be identical to the cattle isolates Londiani, 2490 and the Cameroons. This must cast doubt on the sheep being the major natural host of the 0240/KSGP as it has all the characteristics of an isolate from cattle and it revives the suspicion that the Kenya LSD outbreak was initiated by the introduction of infected sheep (Burdin & Prydie, 1959). The fact that this isolate is in essence a cattle type isolate also raises another interesting point. The KSGP sample used in the present work has been adapted by tissue culture and has a low virulence in cattle yet it appears to be identical on the basis of *Hind*III restriction endonuclease digest patterns to the virulent Londiani cattle isolate. The low number of passages in the lamb testis cells has produced no detectable change at this level of analysis or, in the case of restriction digest patterns using *Bam* HI or *Pst* I (D. N. Black and P. P. Bhat, unpublished observations). However, whatever change has occurred it has attenuated the virus with respect to its virulence towards cattle. This problem is at present being studied further.

As discussed the three Kenya sheep isolates, measured by their *Hind*III restriction digest patterns, are very similar. However, when these patterns are compared with those of typical sheep isolates from other countries, e.g. Nigeria, India and Iraq (Black, Hammond & Kitching, 1986, Gershon & Black, 1988) larger differences are observed. In fact, the Kenyan isolates are more closely related to capripoxvirus isolates from Oman (sheep) and the Yemen (goat) which have been shown to represent a group of capripoxviruses which have probably arisen through the recombination between cattle and goat capripoxvirus isolates (Black, Hammond & Kitching, 1986, Gershon *et al.* 1988). The presence of this type of capripoxvirus in Southern and Central Africa makes the question of the evolution and epidemiology of the capripoxviruses an extremely important problem, especially when the concept of the use of recombinant poxviruses is being considered as disease control agents.

Despite the very high nucleotide sequence homology of all the capripoxvirus so far studied (Gershon & Black, 1988) we have shown that it is possible to study and compare individual virus isolates by the use of simple restriction endonuclease

digest patterns. The wide application of such studies will greatly enlarge our knowledge of the epidemiology of the capripoxviruses.

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