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Trial of a capripoxvirus-rinderpest recombinant vaccine in African cattle

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SUMMARY

Cattle were vaccinated with differing doses of an equal mixture of capripox-rinderpest recombinant viruses expressing either the fusion protein (F) or the haemagglutinin protein (H) of rinderpest virus. Animals vaccinated with 2×10^4 p.f.u. or greater of the combined viruses were completely protected against challenge, 1 month later, with both virulent rinderpest and lumpy skin disease viruses. Vaccination with any of the doses did not induce any adverse clinical response in the animals or transmission of the vaccine virus between animals. All cattle challenged 6 or 12 months after vaccination with 2×10^5 p.f.u. of the mixture of recombinant viruses were protected from severe rinderpest disease. Ten out of 18 were completely protected while the remaining 8 developed mild clinical signs of rinderpest. Cattle vaccinated with the recombinant vaccines after prior infection with the parental capripox virus showed more marked clinical signs of rinderpest after challenge with virulent rinderpest, but 9 out of 10 recovered, compared with 80% mortality in the unvaccinated controls.

INTRODUCTION

Rinderpest and capripox remain diseases of major economic importance in many areas of the world [1]. At present effective vaccines are available for control of these diseases, but the provision of a cold chain remains a problem in some geographical areas. While improved techniques of freeze-drying have increased the thermal stability of the conventional rinderpest vaccine [2, 3] the advent of recombinant DNA technology makes it possible to design vaccines which will overcome the disadvantages associated with current conventional vaccines and which offer the potential benefits to be gained from the use of multivalent vaccines which protect against several diseases. Several poxvirus recombinant vaccines, which express immunogenic proteins from virus genes, have been shown to be effective in protecting animals against challenge with the respective virus [4], including rinderpest [5–7]. In particular, the value of recombinant vaccines has been clearly demonstrated by the protection of foxes in Europe by a vaccinia virus expressing the rabies virus glycoprotein [8, 9].

The availability of an effective live capripox virus vaccine [10] with a host range which appears to be restricted to cattle, sheep and goats makes capripoxvirus a suitable vector to provide effective multi-valent vaccines for veterinary use. The construction and testing of two capripoxvirus-rinderpest recombinant vaccines, one expressing the rinderpest haemagglutinin (H) protein and the other the rinderpest fusion (F) protein, has previously been described [11–13]. Here we report the results of initial trials of recombinant capripox virus vaccines in indigenous Kenyan cattle under contained field conditions.

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MATERIALS AND METHODS

Vaccine

The vaccine consists of an equal mixture of two components; one a capripoxvirus recombinant which expresses the rinderpest haemagglutin (H) gene, the other a capripoxvirus recombinant which expresses the rinderpest fusion protein (F) gene [11–13].

Lyophilized capripox-rinderpest-F and -H vaccines at 10^6 p.f.u. per vial were stored separately at −20 °C. The vaccines were reconstituted in 1·0 ml sterile phosphate buffered saline (pH 7·4) and mixed in equal amounts. Titres of vaccine referred to in the test relate to the total infectious virus in the mixture. To mimic prior exposure to capripox, selected animals were vaccinated with the KS-1 vaccine strain of capripoxvirus produced at the Pirbright Laboratory.

Challenge viruses

Challenge viruses were virulent Kabete type 'O' rinderpest virus and lumpy skin disease virus (LSDV). The isolate K1167/93 of the latter was used for the 1 month efficacy trial and the Neethling isolate for the 6 and 12 month trials. 10^4 $TCID_{50}$ of Kabete type 'O' and 2×10^6 $TCID_{50}$ of LSDV were used for challenge by the subcutaneous route.

Animals

All the cattle used in this study were of the small East African Zebu breed aged between 1 and 2 years at vaccination. Prior to vaccination they were tested and shown to be free of antibodies to both capripox and rinderpest viruses by virus neutralization tests (VNT) (see below). For the vaccination and the virus challenges the animals were housed in the secure animal facility at the National Veterinary Research Centre at Muguga (Kenya).

Vaccination

Protective dose and 1 month challenge

Vaccine dosages containing either 5×10^5, 2×10^5, 2×10^4 or 2×10^3 p.f.u. of the recombinant virus mixture were prepared and kept on ice prior to use and throughout the vaccination procedure. Four groups, each consisting of 4 animals, were inoculated with 1 of the 4 different doses of the vaccine and 8 unvaccinated control animals were housed, 2 per group, with the vaccinated groups. One of each pair of unvaccinated control animals was used for the rinderpest virus challenge and the other for the capripox virus challenge (Table 1). Clinical examinations were made and rectal temperatures taken daily up to 35 days post-vaccination. Serum samples were collected just prior to vaccination and on 7, 14, 21 and 35 days post-vaccination.

Table 1. *Protective dose of a recombinant capripox/rinderpest vaccine in cattle against lethal challenge of rinderpest (RPV) and lumpy skin disease (LSDV) viruses*

Vaccine dose (p.f.u.)*	Number challenged	RPV (D/T)†	LSDV (LP/T*)‡
5×10^5	4	0/4	0/4
2×10^5	4	0/4	0/4
2×10^4	4	0/4	0/4
2×10^3	4	2/4	0/2‖
Unvaccinated controls	8§	4/4	4/4

* Vaccine comprised equal amounts (p.f.u.) of the CPV-RPV-H and CPV-RPF-F recombinant viruses.
† D/T, number dead/total.
‡ LP/T, number with lesions or pyrexia/total.
§ Four animals challenged with each of RPV and LSDV.
‖ Only two animals survived the RPV challenge.

Six and 12 month challenges

For the 6 and 12 month challenges two independent cohorts of animals were vaccinated at the same time using the following protocol. For each challenge cohort five cattle were vaccinated with the Pirbright capripox vaccine (KS-1). One month later these 5 cattle and an additional 15 animals were vaccinated with 2×10^5 p.f.u. of the combined recombinant viruses and housed in 5 groups of 4 animals. Ten unvaccinated control animals were housed, two per group, with the vaccinated animals. Clinical examinations were made and rectal temperatures taken daily up to 35 days post vaccination. Serum samples were collected just prior to vaccination and on 7, 14, 21 and 35 days post vaccination. One month after the second vaccination the animals were transferred to secure pasture.

Virus challenge

Determination of effective immunizing dose

Thirty-five days after vaccination each group of four vaccinated animals together with one control unvaccinated animal were challenged with a sub-

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cutaneous (s/c) inoculation of 10^4 $TCID_{50}$ of virulent Kabete 'O' rinderpest virus. Prior to the challenge the remaining unvaccinated control animal of each group was removed to a separate box within the isolation compound to act as a sentinel animal for possible disease transmission between boxes in the compound and to await the challenge with LSD virus. Clinical examinations were made and rectal temperatures taken daily. Serum samples were collected on 0, 7, 14, 21, 43, 50 and 64 days post challenge. At 78 days post vaccination the vaccinated cattle which survived challenge with rinderpest virus and the second group of unvaccinated control cattle were challenged s/c with LSD virus. Clinical examinations and samples were collected as described for the rinderpest virus challenge.

Six and 12 months

The 5 animals vaccinated first with the KS-1 vaccine and subsequently with the recombinant capripoxvirus, 10 animals vaccinated with the recombinant capripox viruses alone and 5 unvaccinated control animals were challenged with a s/c inoculation of 10^4 $TCID_{50}$ of virulent Kabete 'O' rinderpest virus. The remaining 5 animals vaccinated with the recombinant capripox virus and 5 unvaccinated control animals were challenged s/c with 2×10^6 $TCID_{50}$ of LSD (Neethling) virus. Clinical examinations were made and rectal temperatures were taken daily. Serum samples were taken pre-challenge and 28 days post-challenge.

Neutralization assays

Rinderpest antibody assays were carried out as described previously [11] using the RPV-RBOK strain. The microneutralization titres of the sera were assayed using flat-bottomed 96-well plates. 50 μl containing 100 $TCID_{50}$ of the RBOK strain of RPV were incubated at 37 °C for 1 h with an equal volume of twofold dilutions of heat-inactivated (56 °C for 30 min) test serum. Indicator Vero cells (25000 cells in 100 μl volume) were then added to each well and the plates incubated at 37 °C. The test was read on the 7th day and the absence of cytopathic effect (cpe) was taken as evidence that the sera contained neutralizing antibodies.

Lumpy skin disease neutralization tests were carried out in 96-well flat bottomed plates using three wells/dilution as described previously [14]. Briefly, 50 μl of twofold dilutions of heat inactivated test serum were incubated for 1 h with an equal volume of 50–100 $TCID_{50}$ LSD K 1167/93 virus. Lamb testis indicator cells (10^3 in 100 μl) were added to each well and the plates incubated at 37 °C. The test was read on day 10, when confluent cell sheets were taken as evidence that the test serum contained LSDV neutralizing antibodies.

RESULTS

Determining of immunizing dose

The results of the 1 month challenge to determine the effective dose for vaccination are summarized in Tables 1 and 2. After vaccination with the recombinant capripoxvirus vaccine there were no local or systemic reactions. Over the 35 day period between vaccination and challenge only very low serum neutralizing titres to LSDV and low to medium serum neutralizing titres to RPV developed (Table 2). All cattle vaccinated with 2×10^4 p.f.u. or more of the recombinant capripoxvirus mixture were fully protected against challenge with the virulent Kabete O RPV strain. One animal (329) which received 5×10^5 developed pyrexia following RP challenge but showed no other disease signs. On challenge with RPV 2 (313, 319) of 4 cattle vaccinated with 2×10^3 p.f.u. of the recombinant capripoxvirus mixture and all 4 of the unvaccinated control animals developed severe clinical signs of rinderpest, as indicated by the elevation in rectal temperatures, nasal discharge and mouth lesions, and died between 12 and 15 days after challenge (Tables 1 and 2). All vaccinated animals which survived the RPV challenge were fully protected against challenge with LSDV, whereas the four unvaccinated control cattle developed pyrexia, lymphadenitis, and papules at the site of virus inoculation 8–10 days post challenge. After challenge with RPV all surviving animals developed very high levels of serum neutralizing antibodies (> 1024), but after LSDV challenge there were only small increases in serum neutralizing antibody titres (from 8–16 before to a maximum of 64 after challenge; Table 2).

On the basis of these results it was decided to vaccinate animals for the long-term protection experiment with 2×10^5 p.f.u. of capripoxvirus-rinderpest viruses.

Challenges after 6 and 12 months

The results of challenges with virulent RP and LSD viruses of cattle 6 or 12 months after vaccination with

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Table 2. *Individual LSDV and RPV neutralizing antibody titres (expressed as the highest dilution giving complete neutralization of 100 $TCID_{50}$) in cattle vaccinated with the capripox/rinderpest recombinant virus vaccine and challenged with virulent RP and LSD viruses at 37 and 78 days post-vaccination respectively*

		Day after vaccination				Day after challenge											
Vaccine dose p.f.u.	Cattle ID number	0		35		7		14		21		43		50		64	
		LSDV	RPV	LSDV	RPV	LSDV	RPV	LSDV	RPV	LSDV	RPV	LSDV	RPV	LSDV	RPV	LSDV	RPV
5×10^5	328	< 4	< 4	16	128	32	8192	16	8192	16	8192	16	8192	32	8192	16	> 8192
	316	< 4	< 4	32	8	32	1024	32	2048	16	2048	16	2048	32	4096	32	8192
	331	< 4	< 4	8	64	16	1024	8	2048	8	2048	8	512	16	2048	16	2048
	329	< 4	< 4	4	8	8	1024	8	8192	8	8192	8	8192	8	8192	32	> 8192
2×10^5	324	< 4	< 4	8	32	16	2048	8	> 8192	16	8192	16	4096	16	8192	16	> 8192
	317	< 4	< 4	8	256	8	8192	16	> 8192	16	8192	8	8192	8	8192	16	> 8192
	314	< 4	< 4	8	16	32	512	16	4096	8	4096	8	2048	32	2048	32	4096
	312	< 4	< 4	8	64	32	8192	16	8192	32	8192	16	8192	32	> 8192	32	> 8192
2×10^4	309	< 4	< 4	8	8	16	4096	16	8192	8	8192	8	> 8192	16	> 8192	16	> 8192
	310	< 4	< 4	16	256	32	4096	16	4096	16	4096	8	8192	32	8192	64	8192
	327	< 4	< 4	8	128	16	2048	8	> 8192	8	8092	8	> 8192	16	> 8192	32	4096
	308	< 4	< 4	8	512	16	4096	16	> 8192	8	> 8192	8	4096	16	4096	32	4096
2×10^3	319	< 4	< 4	4	4	8	< 4	Dead	—	—	—	—	—	—	—	—	—
	313	< 4	< 4	16	4	8	< 4	Dead	—	—	—	—	—	—	—	—	—
	330	< 4	< 4	32	16	32	128	32	> 8192	16	< 8192	16	1024	32	> 8192	32	> 8192
	332	< 4	< 4	8	128	16	512	16	1024	8	1024	16	1024	16	2048	64	2048
RPV	333	< 4	< 4	< 4	< 4	< 4	< 4	Dead	—	—	—	—	—	—	—	—	—
controls	325	< 4	< 4	< 4	< 4	< 4	< 4	Dead	—	—	—	—	—	—	—	—	—
	318	< 4	< 4	< 4	< 4	< 4	< 4	Dead	—	—	—	—	—	—	—	—	—
	315	< 4	< 4	< 4	< 4	< 4	< 4	Dead	—	—	—	—	—	—	—	—	—
LSDV	B17	< 4	< 4	—	—	—	—	—	—	—	—	< 4	< 4	< 4	< 4	16	—
controls	B26	< 4	< 4	—	—	—	—	—	—	—	—	< 4	< 4	< 4	< 4	32	—
	B41	< 4	< 4	—	—	—	—	—	—	—	—	< 4	< 4	< 4	< 4	16	—
	B33	< 4	< 4	—	—	—	—	—	—	—	—	< 4	< 4	< 4	< 4	32	—

Table 3. *Individual RPV neutralizing antibodies (expressed as the highest dilution giving complete neutralization of 100 $TCID_{50}$) in cattle vaccinated with the capripox/rinderpest recombinant viruses and challenged with virulent RPV 6 months and 12 months after vaccination*

Vaccination category: Cattle ID number	Clinical* response	Incubation period (days)	AGID† result (days + ve)	Pre-challenge RP antibody titre	Day 28 post-challenge antibody titre	Protection (%)	
						Complete	Partial
6 month cohort							
CPV/RPVFH							
430	NIL	—	-ve	48	32		
433	+R	4	3	4	256		
434	NIL	—	-ve	64	64		
438	+R	5	3	12	⩾ 4096	50	50
444	NIL	—	-ve	64	48		
461	+R	6	3	6	2048		
462	+R	4	3	8	⩾ 4096		
469	NIL	—	3	64	24		
KS1-CPV/RPVFH							
B17	+R	4	NT	0	2048		
B33	+D	4	-ve	0	D		
B41	+R	3	-ve	0	⩾ 4096	0	80
432	+R	7	4	0	⩾ 4096		
445	+R	6	4	4	⩾ 4096		
Unvaccinated controls							
439	+R	3	1, 3, 4	0	⩾ 4096		
459	+D	3	1, 2, 3	0	D		
482	+D	3	1, 2	0	D	0	20
485	+D	3	1, 2, 3, 4	0	D		
489	+D	3	3, 4	0	D		
12 month cohort							
CPV/RPVFH							
413	NIL(?)	—	—	24	1024		
418	+R	4	—	12	⩾ 4096		
427	+R	4	—	8	⩾ 4096		
441	NIL(?)	—	—	128	1024		
466	NIL	—	—	32	256	60	40
453	NIL	—	—	1024	256		
457	+R	4	—	8	⩾ 4096		
460	+R	5	—	4	⩾ 4096		
471	NIL	—	—	48	512		
473	NIL(?)	—	—	48	64		
KS1-CPV/RPVFH							
414	+R	5	—	8	2048		
428	+R	3	—	4	⩾ 4096		
458	+R	4	—	8	⩾ 4096	0	100
465	+R	3	—	6	⩾ 4096		
490	+R	3	—	2	2048		
Unvaccinated controls							
442	+D	3	—	0	D		
463	+R	3	—	2	⩾ 4096		
448	+D	4	—	4	512	0	20
472	+D	3	—	0	D		
545	+D	3	—	< 4	D		

* +R, recovered; +D, animal died; NIL(?), oculo-natal discharge but no fever.
† AGID, Agar gel immunodiffusion test.

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Table 4. *Individual LSDV neutralizing antibody titres (expressed as the highest dilution giving complete neutralization of 100 $TCID_{50}$) in cattle vaccinated with the capripox/rinderpest recombinant virus vaccine and challenged with virulent LSDV at 6 and 12 months respectively*

Vaccination category cattle ID no.	Clinical* response	Incubation period (days)	DTH† response (hours + ve)	Pre-challenge LSDV anti-body titre	Day 28 post-challenge antibody titre
6 month cohort					
CPV/RPVFH					
494	−ve	—	48–96 (co)	4	16
435	−ve	—	24–96 (co)	4	4
452	−ve	—	24–96 (co)	< 4	16
426	−ve	—	48–96 (co)	4	32
447	−ve	—	48–96 (co)	4	16
Unvaccinated controls					
464	+	5	NIL	< 4	8
474	+G	5	NIL	< 4	32
492	+	7	NIL	< 4	8
478	+	5	NIL	< 4	16
495	+	9	NIL	< 4	4
12 month cohort					
CPV/RPVFH					
455	−ve	—	48–96 (co)	8	NT
417	−ve	—	24–96 (co)	4	NT
475	+G	5	NIL	< 4	NT
483	−ve	—	48–96 (co)	4	NT
437	−ve	—	48–96 (co)	4	NT
Unvaccinated controls					
476	+G	5	NIL	< 4	NT
546	+?	4	NIL	< 4	NT
488	+	10?	NIL	< 4	NT
550	+G	5	NIL	< 4	NT
486	—	5	NIL	< 4	NT

* +, clinical response observed with development of a lump 1·5–3 cm in diameter at the site of intradermal inoculation and prescapular lymph node enlargement; +G, generalized LSD lumps on neck region, dependent oedema and recovered; +?, prescapular lymph node enlargement was the only clinical sign observed.
† DTH, delayed type hypersensitivity at intradermal inoculation site within 48 hours; co, circumscribed and oedematous swelling.

2×10^5 p.f.u. of the recombinant capripox/rinderpest viruses and cattle vaccinated with the mixture of recombinant viruses after prior exposure to capripoxviruses are shown in Tables 3 (RPV challenges) and 4 (LSDV challenges). In both the 6 and 12 month challenges 4 of the 5 unvaccinated controls animals died following the RPV challenge and the 5th animal of each group developed pyrexia and oculo-nasal secretion characteristic of rinderpest. In comparison, 4 out of 8 vaccinated animals in the 6 month RPV challenge and 6 out of 10 vaccinated animals in the 12 month RPV challenge showed no clinical signs of rinderpest other than a small oculo-nasal discharge in 3 animals (413, 441, 473). The remaining four vaccinated animals in each cohort showed mild rinderpest. All animals that had been vaccinated following exposure to capripoxvirus in both the 6 and 12 month challenges developed clinical signs of rinderpest to varying degrees and one animal (B33, Table 3) in the 6 month challenge died. In

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contrast 8 out of 10 unvaccinated control animals died following challenge with virulent RPV.

In the 6 month challenge cohort the four fully protected vaccinated animals had low to medium levels (48–64) of anti-RPV serum neutralizing antibodies at the time of challenge, which did not rise significantly after challenge with virulent RPV. In contrast, the four partially protected vaccinated cattle had very low titres of serum neutralizing antibodies (4–12) prior to challenge and showed a marked anamnestic response upon challenge. A similar situation was observed for the 12 month cohort (Table 3).

Challenges with virulent LSDV at 6 months and 12 months following vaccination of animals with the recombinant virus resulted in 5/5 and 4/5 animals, respectively, being fully protected. In addition they showed a delayed the hypersensitivity at the site of inoculation 24–96 h after challenge. The fifth vaccinated animal (475; Table 4) in the 12 month challenge failed to show a DTH response and developed generalized lumps in the neck region, dependent oedema, and recovered. Because this animal also showed no pre-challenge LSDV neutralizing antibodies it is possible that it was not vaccinated. In comparison all five unvaccinated control animals in both groups showed no DTH response and developed clear and severe signs of lumpy skin disease.

DISCUSSION

The principal aim of this work was to test the capripox-rinderpest recombinant vaccines in African breeds of cattle for innocuity and efficacy. From the data presented here and earlier [11–13] it can be seen that the vaccines behave in a similar way irrespective of the cattle breed – African Zebu or European Holstein/Friesian. The results of the effective dose trial in Kenyan cattle showed that 1 month post-vaccination the cattle were fully protected against challenge with lumpy skin disease and rinderpest as had previously been found with European cattle. On the other hand, cattle challenged with RPV 6 months or 1 year after vaccination showed a mixed response; over half being fully protected while the remainder showed mild signs of disease. None of the cattle vaccinated after prior exposure to capripoxvirus were fully protected from rinderpest and one died. These results are in marked contrast to those observed using a recombinant vaccinia-rinderpest virus expressing only the H gene. With this vaccine full protection was observed up to 12 months following vaccination of naive animals and 1 month following vaccination with prior exposure to non-recombinant vaccinal virus [15, 16].

There are four aspects of this work that might explain the differences noted above; genetic status of the vaccinated animals, differential growth of vaccinia and capripoxviruses in cattle, the dose of the vaccine used and differences in the promoters used to control rinderpest gene expression in the recombinant viruses.

The responses of different breeds of cattle to infection is well documented. The trials of the recombinant vaccinia-rinderpest vaccine used European Holstein Freisian cattle whereas in the present work African Zebu cattle were used. The observed differences in the long term challenge trials could be due to the genetic differences between these two types of cattle. Vaccinia virus is known to replicate readily in a wide range of animal hosts including cattle. It is possible that the capripoxvirus recombinant vaccine grows less well than the vaccinia recombinant vaccine and that this may lead to a lower immune response to the capripoxvirus vaccine. This could explain the reduced long term immunity to rinderpest in the animals vaccinated with the capripoxvirus-recombinant vaccine. However, the vaccinia-rabies recombinant vaccine which exhibits minimal replication in foxes [17] has been shown to provide solid protection against rabies for at least 3 years and its use in Europe has led to the elimination of fox rabies in Belgium [8, 9].

Previous reports of effective long term protection using recombinant poxvirus vaccines have used considerably higher doses of vaccine (e.g. 10^7–10^8 p.f.u.) than that used in this study (2×10^5 p.f.u.) [16, 18]. We chose a dose which was ten times the minimum effective dose at 1 month and which is a realistic dose for practical field use. It is possible, however, that a dose greater than 2×10^5 p.f.u. may be necessary to stimulate long term immunity to rinderpest. A final difference between the vaccinia and capripoxvirus recombinant vaccines, which may have an important bearing on the different responses of cattle to challenge. The vaccinia recombinant uses an 'early–late' poxvirus promoter for the expression of the rinderpest virus protein whereas the capripoxvirus-recombinant vaccine uses a 'late' poxvirus promoter. Genes under the control of the early promoter are expressed soon after infection whereas those under the control of late promoters are expressed later in the infection cycle after the virus has

replicated its genome. Evidence indicates that proteins expressed under the control of early poxvirus promoters tend to induce cellular mediated immunity whereas those expressed under the control of late poxvirus promoters induced humoral immunity [19]. A recombinant vaccine using an 'early-late' promoter might induce a more pronounced cellular immune response and would be expected to result in a long lasting immunity. The 'late' promoter used in the recombinant capripox-rinderpest recombinant vaccine would be expected to induce a more humoral based immunity which may be much less effective and shorter lived. It should be noted, however, that this vaccine completely protected cattle against lumpy skin disease 12 months after vaccination demonstrating that the recombinant vaccine induced long term immunity against the homologous challenge. This can be tested by using a recombinant capripox-rinderpest vaccine which incorporates an 'early–late' poxvirus promoter and this work is currently underway.

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