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SHORT PAPER
Long term immunity in African cattle vaccinated with a recombinant capripox-rinderpest virus vaccine

C. K. NGICHABE[1], H. M. WAMWAYI[1], E. K. NDUNGU[1], P. K. MIRANGI[1], C. J. BOSTOCK[2], D. N. BLACK[2]* AND T. BARRETT[2]†

[1] *Kenya Agricultural Research Institute, National Veterinary Research Centre Muguga, P.O. Box 32 Kikuyu, Kenya*
[2] *Department of Molecular Biology, Institute for Animal Health, Pirbright Laboratory, Pirbright, Woking, Surrey, GU24 0NF, UK*

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SUMMARY

Cattle were vaccinated with a recombinant capripox-rinderpest vaccine designed to protect cattle from infection with either rinderpest virus (RPV) or lumpy skin disease virus (LSDV). Vaccination did not induce any adverse clinical responses or show evidence of transmission of the vaccine virus to in-contact control animals. Approximately 50% of the cattle were solidly protected from challenge with a lethal dose of virulent RPV 2 years after vaccination while at 3 years approx. 30% were fully protected. In the case of LSDV, all of 4 vaccinated cattle challenged with virulent LSDV at 2 years were completely protected from clinical disease while 2 of 5 vaccinated cattle were completely protected at 3 years. The recombinant vaccine showed no loss of potency when stored lyophylized at 4 °C for up to 1 year. These results indicate that capripoxvirus is a suitable vector for the development of safe, effective and stable recombinant vaccines for cattle.

Rinderpest virus (RPV) is a member of the genus *Morbillivirus* in the family *Paramyxoviridae* and the virus is most closely related to human measles virus [1]. It is responsible for an economically important disease of domestic cattle and buffalo and is also highly virulent in some wild ruminant species [2]. A new generation of vaccines, based on the expression of foreign immunogens by recombinant poxviruses, is being used successfully to control virus diseases of veterinary importance. A vaccinia recombinant expressing the rabies glycoprotein is being used to control rabies in wildlife in Europe and the USA [3, 4], while a fowlpox recombinant expressing Newcastle disease virus glycoprotein genes can protect chickens against this economically important disease [5]. Recombinant poxviruses that express either the haemagglutinin (H) or fusion (F) protein genes of RPV have been shown protect cattle against a lethal virus challenge [6–9]. A bi-valent vaccine, designed to protect against two economically important virus diseases of cattle, RPV and lumpy skin disease virus (LSDV), was constructed by inserting the H gene and the F gene of RPV into the attenuated Kenya sheep poxvirus vaccine [10]. This recombinant vaccine has been shown to protect cattle both against RPV and lumpy skin disease virus (LSDV) for up to 1 year following a single vaccine inoculation [11]. Here we report on the long-term efficacy, up to 3 years following vaccination. The stability of this vaccine on storage was also investigated.

All the cattle used in this study were African Zebu breed aged between 1 and 1·5 years at vaccination. Prior to vaccination they were tested and shown to be free of antibodies to both RPV and LSDV by virus

* Present address: Wright Fleming Institute, Department of Infectious Diseases, Imperial College Medical School, Norfolk Place, London W21PG, UK.
† Author for correspondence.

neutralization tests. The vaccine consisted of an equal mixture of two components; one a capripoxvirus recombinant expressing the rinderpest H protein, the other, a capripoxvirus recombinant expressing the rinderpest F protein [6, 7]. Lyophilized capripox/rinderpest-H and -F vaccines were stored separately at −20 °C at concentrations of 10^6 $TCID_{50}$/vial. The vaccines were reconstituted in 1·0 ml of sterile PBS at pH 7·4, mixed in equal volumes, and diluted with PBS to obtain the required dosage. This mixture of capripox/rinderpest-H and -F viruses is subsequently referred to as the recombinant vaccine. A dose of $10^{5·3}$ $TCID_{50}$/ml of the recombinant vaccine, determined previously as an effective dose [11], was injected subcutaneously in the shoulder region of each animal. To mimic prior field exposure to LSDV, selected animals were vaccinated with the normal capripox vaccine (KS-1) [10], the virus strain used to produce the recombinants, 1 month prior to vaccination with the recombinant vaccine. Following vaccination, all the animals were observed daily for clinical signs of disease and their rectal temperatures recorded for up to 28 days. During vaccination, and also during the subsequent virus challenges, the animals were housed in the secure animal facility at the National Veterinary Research Centre (NVRC) at Muguga, Kenya. After the 28-day period of observation, the cattle were released to graze in confined paddocks within the quarantine area of NVRC.

Two years after vaccination the first group of cattle was challenged with virulent RPV. The rinderpest challenge virus was 10^4 $TCID_{50}$ of the virulent 'Kabete O' strain in a 1·0 ml volume injected subcutaneously in the shoulder region. Following challenge clinical examinations were performed daily and the rectal temperatures recorded. Serum samples were collected on days 0, 7, 14, 21 and 28 post challenge (d.p.c). The 2-year group consisted of 11 cattle that had received only the recombinant vaccine and 5 that had been vaccinated with the recombinant vaccine after prior exposure to the KS1 vaccine. Four unvaccinated control animals were challenged at the same time. Seven out of 11 vaccinated cattle were protected from severe clinical rinderpest, 6 showing complete protection and 1 was partially protected. In the pre-exposed group 3 out of 5 vaccinated animals were protected, 2 were completely protected and 1 developed mild clinical signs of disease. The remaining 6 cattle developed typical rinderpest, the clinical signs being fever, diarrhoea, congestion of the ocular, nasal and oral mucosae. Three showed very severe signs and were killed. Two of them had been vaccinated with the recombinant vaccine alone while the other had been pre-vaccinated with KS-1 vaccine. All 4 unvaccinated control cattle also developed severe rinderpest and were killed. Roughly the same percentage of cattle in the group which received only the recombinant vaccine and in the group pre-vaccinated with KS-1 showed moderate signs of rinderpest infection on challenge and recovered; 36% and 40%, respectively. There were more fully protected cattle in the group that received only the recombinant vaccine (55%) compared to the group pre-vaccinated with KS-1 (40%). The results of the challenge experiments with virulent RPV at 2 years post vaccination with the recombinant vaccine are shown in Table 1.

Not all of the vaccinated cattle survived for 3 years, some having died from causes unrelated to rinderpest or LSD infections. As a result there were only 6 remaining vaccinated cattle at 3 years and these were first challenged with virulent RPV. Five of the 6 were protected from severe clinical disease; 2 being completely protected while 3 developed only a mild form of the disease. The sixth animal developed severe clinical rinderpest and was killed. Pre-challenge RPV antibody titres were very low (2–4) in the animals which were not fully protected but they showed very high anamnestic responses following challenge. The two fully protected cattle had reasonably high pre-challenge antibody titres to rinderpest (48 and 96) but they also showed a fourfold or greater anamnestic response to RPV on challenge, indicating replication of the challenge virus. Although the numbers were too small to draw statistically valid conclusions, at 3 years the majority of animals were in the partially protected group, indicating a gradual loss of protective immunity. The results of the challenge experiments with virulent RPV at 3 years post vaccination with the recombinant vaccine are shown in Table 1.

A separate group of vaccinated animals were challenged with LSDV at 2 years. The challenge virus was 10^6 $TCID_{50}$ of the virulent Neethling strain in a 1·0 ml volume, 0·8 ml being injected intravenously and 0·2 ml injected intradermally. Following challenge clinical examinations were performed daily and the rectal temperatures recorded. Serum samples were collected on 0, 7, 14, 21 and 28 d.p.c. A delayed type hypersensitivity (DTH) reaction was seen in all vaccinated animals after challenge, indicating prior exposure to the virus, and all were protected from clinical signs characteristic of lumpy skin disease. The pre-challenge LSDV neutralizing antibodies were low

Table 1. *Individual RPV neutralizing antibody titres (expressed as a reciprocal of the highest dilution giving complete neutralization of 100 $TCID_{50}$) in cattle vaccinated with the capripox-rinderpest recombinant virus vaccine and challenged with virulent RPV at 2 and 3 years post vaccination respectively*

Vaccination category/cattle IDNo	Clinical response (protection)*	Incubation period (days)	Prechallenge RPV antibody titre	Day 28 post challenge RPV antibody titre	Protection (%)		
					Complete	Partial	None/fatal
2 year cohort							
CPV.RPV.FH							
411	+R (none)	4	4	256			
415	+D (none)	4	2	D			
425	NIL (full)	—	8	> 4096			
436	NIL (full)	—	48	768			
451	NIL (full)	—	16	64	55	9	36
477	NIL (full)	—	4	64			
487	NIL (full)	—	16	768			
497	NIL (full)	—	6	768			
542	+D* (none)	—	4	D			
544	+R (partial)	6	8	> 4096			
554	+R (none)	4	0	> 4096			
KS1+CPV.RPV.FH							
449	+R (none)	3	0	512			
467	NIL (full)	—	8	1024			
484	+D (none)	3	0	D	40	20	40
496	+R (partial)	3	4	3072			
498	NIL (full)	—	2	256			
Unvaccinated controls							
555	+D (none)	3	0	D			
561	+D (none)	3	0	D			
564	+D (none)	3	0	D	0	0	100
565	+D (none)	4	0	D			
3 year cohort							
CPV.RPV.FH							
416	NIL (full)	—	96	384			
419	+R (partial)	4	2	⩾ 4096			
431	+D (none)	3	2	D	33	50	17
481	NIL (full)	5	48	242			
553	+R (partial)	6	2	⩾ 4096			
557	+R (partial)	4	4	⩾ 4096			
Unvaccinated controls							
C 6	+D	3	3	D			
C14	+D	3	< 2	D	0	0	100
C16	+D	3	< 2	D			

* **Nil (Full): Full protection** – no clinical signs other than slight oculo-nasal discharges, **+R (Partial): Partial protection** – slight fever, ocular, oral and nasal congestion and no other clinical signs, **+R (None): No protection** – Fever lasting 1–6 days, ocular and nasal congestion and subsequent discharges, dull, excessive salivation, diarrhoea, dehydration, recumbency, inappetant leading to recovery. **+D (None): No protection** – Fever lasting 1–6 days, ocular and nasal congestion and subsequent discharges, dull, excessive salivation, diarrhoea, dehydration, recumbency, inappetant. Most of these animals were euthanased at this stage of the disease but a few died naturally. Serology: The RPV virus neutralization antibody assays were carried out as described previously [11].

but all animals showed a great increase in LSDV neutralizing titres when tested 28 d.p.c. In contrast, five unvaccinated control animals failed to show a DTH response on challenge with virulent LSDV and developed mild clinical signs of lumpy skin disease and recovered. The results of the challenge experiments with virulent LSDV at 2 years post vaccination are shown in Table 2. For the 3-year LSDV challenge,

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Table 2. *Individual LSDV neutralizing antibody titres (expressed as a reciprocal of the highest dilution giving complete neutralization of 100 $TCID_{50}$) in cattle vaccinated with the recombinant capripox-rinderpest virus vaccine and challenged with virulent LSDV at 2 and 3 years post vaccination respectively*

Vaccination category/cattle IDNo	Clinical response (protection)*	Incubation period (days)	DTH response (hours +ve)	Prechallenge LSDV antibody titre	Day 28 post challenge LSDV antibody titre	Protection (%)		
						Complete	Partial	None
2 year cohort								
CPV.RPV.FH								
548	NIL (full)	—	24 (co)	0	64			
449	NIL (full)	—	24 (co)	6	192	100	0	
558	NIL (full)	—	48 (co)	2	48			
560	NIL (full)	—	48 (co)	6	192			
Unvaccinated controls								
871	+ (none)	5	NIL	0	2			
872	+ (none)	5	NIL	0	2			
873	+ (none)	8	NIL	2	8	0	0	100
874	+ (none)	9	NIL	NT	NT			
875	+ (none)	7	NIL	NT	NT			
3 year cohort								
CPV.RPV.FH								
416	+R (partial)	5	NIL	0	20			
419	NIL (partial)	5	NIL	0	0	40	60	0
481	NIL (full)	—	48 (co)	0	20			
553	NIL (full)	—	48 (co)	0	48			
557	+R (partial)	—	NIL	0	0			
Unvaccinated controls								
066†	NIL (full)	4	48	0	8			
062	+ (none)	5	NIL	0	80			
063	+ (none)	5	NIL	2	NT	20	0	80
060	+ (none)	5	NIL	0	20			
065	+ (none)	4	NIL	0	32			

* **NIL (Full): Full protection** – No clinical signs except for transient pyrexia (39 °C or 40 °C) post challenge, circumscribed and edematous swelling (Co) within 48 h, **+R (Partial): Partial protection** – transient fever, slight lymphnode enlargement with no circumscribed edematous swelling within 48 h, **+(None): No protection** – development of a lump (> 1·0 cm in diameter) at the site of challenge in more than 5 days after challenge leading to ulceration and scab formation and enlargement of prescapular lymphnodes with or without pyrexia, DTH delayed type hypersensitivity reaction.
† Animal may have been exposed to LSD but went undetected by the screening serum neutralization test. The RPV LSDV virus neutralization antibody assays were carried out as described previously [11].

the five vaccinated cattle that survived the RPV challenge were subsequently challenged with virulent LSDV. At 3 years none of the vaccinated animals had detectable LSDV neutralizing antibodies prior to challenge and most (3/5) failed to show a DTH response on challenge. The 2 animals showing a DTH response were fully protected against virulent LSDV challenge while the other 3 developed mild clinical signs of lumpy skin disease. Only 3 of the 5 animals had developed LSDV neutralizing antibodies by 28 d.p.c. At the same time 4 of 5 unvaccinated controls developed mild clinical signs of lumpy skin disease and developed neutralizing antibodies to LSDV by 28 d.p.c. One of the control animals was fully protected from LSDV and showed a DTH response. This animal may have been exposed to natural LSDV infection that went undetected in the screening test. The results of the challenge experiments with virulent LSDV at 3 years post vaccination are shown in Table 2. The observation that complete protection against LSDV lasts for up to 2 years in cattle, contrasts with previously published reports on experiments with sheep which showed that immunity conferred by the vector vaccine (KS-1) does not exceed 12 months [12, 13].

The stability of the lyophilized recombinant vaccine

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Table 3. *Clinical and serological responses of cattle to virulent rinderpest virus challenge after vaccination with recombinant capripox-rinderpest vaccine virus stored at +4 °C for 6 and 12 months*

Duration at +4 °C	Vaccination category/cattle IDNo.	Clinical response (protection)*	Incubation period	Pre-challenge RPV antibody	Day 28 post challenge RPV antibody titre	Protection (%)
6 months	CPV.RPV.FH					
	491	NIL (full)	—	144	4096	
	456	NIL (full)	—	256	1024	100
	479	NIL (full)	—	128	384	
	493	NIL (full)	—	96	512	
	Unvaccinated controls					
	773	+D (None)	3	< 4	—	0
	774	+D (None)	3	< 4	—	
12 months	CPV.RPV.FH					
	777	NIL (full)	—	128	512	
	778	NIL (full)	—	64	4096	100
	779	NIL (full)	—	192	512	
	780	NIL (full)	—	256	1024	
	Unvaccinated controls					
	782	+D (None)	3	< 4	—	0
	776	+D (None)	3	< 4	—	

* **NIL (Full): Full protection** – no clinical signs of rinderpest, **+D (None): No protection** – Fever setting in within 3 days, ocular and nasal congestion and subsequent discharges, dull, excessive salivation, diarrhoea, dehydration, recumbency, inappetant. The animals were euthanased at this stage of the disease (+D). The RPV virus neutralization antibody assays were carried out as described previously [11].

when stored +4 °C was also tested. After either 6 or 12 months of storage the vaccine was used to inoculate four cattle. On subsequent challenge, all eight cattle were fully protected from clinical rinderpest. All unvaccinated controls developed severe disease and were killed. The vaccinated cattle showed a threefold, or greater, anamnestic antibody responses to RPV on challenge, again indicating that replication of the challenge virus had occurred in these animals. The results are shown in Table 3.

The present study showed clearly that a single vaccination with the recombinant capripox-rinderpest vaccine was capable of inducing protective immunity that could last for up to 3 years in some vaccinated cattle. A majority of cattle showed significant levels of immunity to rinderpest when challenged at 2 (10/16) and 3 (5/6) years following vaccination. The high anamnestic responses observed on challenge were an indication that the challenge virus had replicated in all of the vaccinated animals. When the RPV challenge was carried out at 2 years there was a slight decrease in the number of fully protected compared to partially protected cattle in the group pre-vaccinated with KS-1 but the overall level of protection from severe disease was approximately the same. This indicated that any pre-existing antibodies resulting from pre-exposure to KS-1 could have only partially interfered with the recombinant vaccine efficacy. Previously we showed that all vaccinated cattle challenged after 6 or 12 months were protected from severe clinical rinderpest [11] and the new results show that a single dose of the vaccine can confer significant levels of immunity to virulent RPV for at least 3 years post vaccination.

A similar long-term vaccine trial has been carried out in European cattle with vaccinia virus recombinant that expresses only the H protein of RPV. This study showed that protective immunity also lasted at least 3 years in some animals. At 3 years all of 6 cattle challenged were protected from severe rinderpest and survived, 2 being fully protected from clinical disease [8]. This is a very comparable result to that from the capripox recombinant trial where, apart from the single animal that developed severe disease and was killed following challenge, 2 out of 6 animals were fully protected from disease in the third year and the rest were partially protected. In the vaccinia recombinant trial a strong anamnestic response was also observed in all of the animals following challenge with virulent RPV, again indicating replication of the

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challenge virus even in the fully protected animals. However, animals vaccinated with the vaccinia recombinant received a 500 fold higher dose (10^8 p.f.u./animal) than those vaccinated with the capripox recombinant ($10^{5·3}$ $TCID_{50}$/animal), indicating that capripox might be a better vector for delivering foreign immunogens to cattle.

The basis of the protective immunity triggered by these recombinant vaccines is not entirely clear but it is likely to be mainly cell-mediated since other studies have shown that neutralizing antibody alone will not protect against RPV challenge. Bassiri et al. [14] showed that administration of RPV H protein produced from a baculovirus recombinant could induce high levels of neutralizing antibody but failed to protect cattle from subsequent challenge with rinderpest. On the other hand cattle vaccinated with baculovirus expressed H protein in combination with ISCOMs, a formulation known to induce cell-mediated responses, were protected from rinderpest challenge [15]. In addition, some capripox recombinant animals had only barely detectable neutralizing antibody titres, yet were protected from a severe form of the disease, indicating the importance of a non-antibody based cell-mediated immune response in protection. Similarly, cross-protection by these recombinant viruses against a related morbillivirus disease of small ruminants, peste des petits ruminants virus (PPRV), was not dependent on neutralizing antibody. The protection given by either the vaccinia/rinderpest or capripox/rinderpest recombinant vaccines occurred in the absence of detectable PPRV-specific neutralizing antibodies [16, 17]. Nevertheless, in the case of both the capripox and vaccinia recombinant vaccines, the animals that were fully protected at 3 years had the highest pre-challenge anti-RPV neutralizing antibody titres, indicating some beneficial protective affects of circulating neutralizing antibodies. It is possible that the higher neutralizing titres in the fully protected cattle may simply reflect a better overall immune response to the vaccine, including a better cell-mediated immune response, in these animals, rather than implicating the antibodies themselves in the protective effect.

The conventional tissue culture rinderpest vaccine [18] confers life-long immunity to vaccinated animals and has been used very successfully to control rinderpest and eradicate the disease from most of the world. The high thermolability of this vaccine was considered one of the major impediments to its use in the field and the rationale for the development of these poxvirus-based recombinant vaccines was to produce a more thermostable rinderpest vaccine. However, improvements in the freeze drying process has greatly increased the stability of the conventional vaccine during storage at high ambient temperatures and this has reduced the urgency to develop more heat stable rinderpest vaccines [19]. Another advantage of recombinant RPV vaccines would be the ability to use them as marked vaccines to identify vaccinated animals and distinguish them from unvaccinated animals that had recovered from natural infection. Rinderpest has now been eliminated from most of the previously endemic regions of the world and vaccination will soon have to cease in countries that are now free of disease [20]. In the small number of countries where rinderpest remains endemic, and where vaccination must continue for some time to come, the use of a marker vaccine would be highly desirable. This would allow serological surveys to be used for the positive detection of circulating disease. Even this advantage, which the poxvirus recombinant vaccines had over the tissue culture attenuated vaccine has been removed since reverse genetics technology has enabled genetic markers to be introduced into the genome of the conventional vaccine [21], although this has yet to be tested under field conditions. The one remaining advantage of the recombinant capripox vaccine is its ability to act as a dual vaccine to protect livestock against more than one disease in a single inoculation. The demonstration of the induction of long-term immunity by the capripox vaccine may make this approach feasible for other diseases where no effective vaccines are yet available.

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