A randomised vaccine field trial in Kenya demonstrates protection against wildebeest-associated malignant catarrhal fever in cattle

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ABSTRACT

Wildebeest-associated malignant catarrhal fever (WA-MCF), a fatal disease of cattle caused by alcelaphine herpesvirus 1 (AlHV-1), is one of the most important seasonal diseases of cattle in wildebeest endemic areas, with annual incidence reaching 10%. Here we report efficacy of over 80% for a vaccine based on the attenuated AlHV-1 C500 strain, in preventing fatal WA-MCF in cattle exposed to natural wildebeest challenge. The study was conducted at Kapiti Plains Ranch Ltd, south-east of Nairobi, Kenya. In 2016, 146 cattle were selected for a randomised placebo-controlled trial. Cattle were stratified according to breed and age and randomly assigned to groups given vaccine or culture medium mixed with Emulsigen®. Cattle received prime and boost inoculations one month apart and few adverse reactions (n = 4) were observed. Indirect ELISA demonstrated that all cattle in the vaccine group developed a serological response to AlHV-1. The study herd was grazed with wildebeest from one month after booster vaccination. Three cattle, two that received vaccine and one control, succumbed to conditions unrelated to WA-MCF before the study ended. Twenty-five cattle succumbed to WA-MCF; four of the remaining 71 cattle in the vaccine group (5.6%) and 21 of the remaining 72 control cattle (29.2%; χ^2 = 13.6, df = 1, p < 0.001). All of the WA-MCF affected cattle were confirmed by PCR to be infected with AlHV-1 and in 23 cases exhibited histopathology typical of WA-MCF. Vaccine efficacy was determined to be 80.6% (95% CI 46.5–93.0%). Hence, the AlHV-1 C500 vaccine is a safe and potentially effective novel method for controlling WA-MCF in cattle. The implementation of this vaccine may have significant impacts on marginalised cattle keeping communities.

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1. Introduction

Wildebeest-associated malignant catarrhal fever (WA-MCF) is a fatal disease of cattle in Africa. It is caused by alcelaphine herpesvirus 1 (AlHV-1), endemic to wildebeest (*Connochaetes taurinus* and *C. gnou*) [1]. Wildebeest do not suffer from disease but viraemic calves excrete the virus onto pasture where it may be spread to cattle during co-grazing [2]. Infection in cattle results in a fatal lymphoproliferative disease, with lymphoid cell accumulation in many tissues, vasculitis, and tissue necrosis particularly of the epithelium [3]. Clinical signs of WA-MCF include pyrexia, inappetence, lymphadenopathy, nasal/ocular discharges and corneal opacity [4]. The acute form of WA-MCF results in severe depression and pain which causes significant animal suffering. The incubation period in cattle is estimated from experimental infections to be 20–50 days and cattle die within 9 days of clinical disease onset [1,5]. Cattle are a dead end host for the disease so infected individuals do not transmit the virus [6]. The case fatality rate of WA-MCF

in cattle is reported to approach 100%, however this rate reflects fatalities following onset of clinical signs and does not include all infected animals, such as PCR or seropositive cattle that did not develop WA-MCF in previous trials [7]. The annual incidence of WA-MCF in at-risk herds is reported to be between 3 and 12% [8] and cattle owners in wildebeest endemic areas report WA-MCF to be one of the most important diseases of cattle [8,9]. The annual incidence of WA-MCF at Kapiti Plains Ranch is between 2 and 9% [10].

WA-MCF is reported in eastern and southern Africa in areas where wildebeest are found [9]. In East Africa disease outbreaks occur shortly after wildebeest calving between March and June annually [9–11]. In Kenya, the areas of highest risk are east and south of Nairobi from the Athi-Kaputiei plains to Amboseli and in the Masai Mara ecosystem bordering Tanzania, a major land mass through which wildebeest migrate [12]. Traditionally, to avoid WA-MCF, pastoral cattle owners move their herds away from wildebeest grazing pastures during the wildebeest calving season [13]. Wildebeest avoidance results in an increase in the costs of production due to extra labour costs, the requirement to graze alternative pastures, often of lower nutritional value, and lost opportunities to utilise milk [13]. Other potential negative impacts include increasing the likelihood of predation of cattle, exposure to other diseases, and decreased access to water [8]. Cattle that develop WA-MCF are sold at ~50% of market value, adding to the economic impact of the disease [8].

As a result of the considerable annual costs associated with WA-MCF, there is a demand from cattle owners living within the WA-MCF risk area for a vaccine [9]. Previous attempts to develop a vaccine using inactivated AlHV-1 virus in cattle were unsuccessful [14]. However, experimental vaccine trials using tissue-culture attenuated AlHV-1 virus under laboratory conditions in Holstein/Friesian cattle have been successful. The attenuated vaccine strain of AlHV-1, derived by serial culture of the C500 viral isolate from a Kenyan MCF case, has provided proof-of-concept for the safety and efficacy of a vaccine for the control of WA-MCF [5,15]. Two field trials with this vaccine in crossbred Tanzanian shorthorn zebu cattle in the Simanjiro Wildlife Dispersal Area of northern Tanzania were conducted over the period 2011 to 2012 [7]. Relative to control cattle, the vaccine reduced detection of AlHV-1 virus by PCR in the blood of vaccinated cattle by 56% [7]. However, because few WA-MCF cases occurred during the trials, vaccine efficacy for reducing disease could not be estimated. It is possible that immunization of both cattle groups against East Coast fever (ECF) immediately prior to the study imparted protection against WA-MCF, thereby compromising the vaccine trial [7]. This potential confounding factor was not previously known. The aim of this study was to assess the AlHV-1 C500 vaccine in Kenya, at a confined field site where cattle and wildebeest co-exist and in an area that is free of ECF.

2. Materials and methods

2.1. Study area and population

The trial was conducted at Kapiti Plains Ranch, Ltd in central Kenya, approximately 70 km south-east of Nairobi (1.633333°S, 37.145267°E). The ranch is 13,000 ha and has approximately 2500 cattle (Boran and Boran/Friesian crosses), 1200 sheep and 250 goats. In addition, wild herbivores including wildebeest, giraffe (*Giraffa camelopardalis tippelskirchii*), hartebeest (*Alcelaphus buselaphus*), zebra (*Equus quagga*) and Thomson gazelle (*Eudorcas thomsoni*) roam freely. Various carnivores, including lion (*Panthera leo*), hyena (*Crocuta crocuta*), leopard (*Panthera pardus*) and cheetah (*Acinonyx jubatus*) are also present on the ranch. There are no fences on the southern border of Kapiti Plains Ranch, allowing the movement of wildlife in and out of the property.

2.2. Animal care and use

The ILRI Institution Animal Care and Use Committee approved the study (IACUC 2016-01). The Directorate of Veterinary Services, Republic of Kenya granted approval for the trial (VACC/I/VOL. XV/79). A clinical scoring system was used to assess disease severity and animal welfare as described previously [15]. Clinical scoring, conducted daily after the onset of fever (temperature > 40 °C), was based on temperature (1 point per day of temperature), diarrhoea (1 point = slight, 2 = moderate, 6 = haemorrhagic), nasal pathology (1 point = opague, 2 = fibrinous) and ocular pathology (1point = clear discharge, 2 = conjunctivitis, 3 = opaque) [15]. Cattle that were diagnosed with WA-MCF and reached a score of 6 were euthanized. The humane endpoint included greater than 3 days of pyrexia (>40 °C), inappetence, severe respiratory distress and/or recumbency.

Cattle were grazed as one group on open pasture during the day with a livestock herder and a research assistant monitoring for clinical signs consistent with WA-MCF. Cattle were confined to the boundaries of Kapiti Plains Ranch. Cattle were corralled at night in a fenced compound with a security officer to prevent theft or predation.

2.3. Vaccination regimen

The study was a blinded randomised placebo-controlled trial. The sample size was calculated for a vaccine efficacy of 56% and an incident proportion in the control (placebo) group of 0.35, with level of significance 5% and power 80% [7,16]. The calculated sample size was 73 cattle in each group.

In January 2016, 146 castrated male cattle (98 Boran and 48 Boran/Friesian cross) aged between 8 and 19 months were selected from the Kapiti Plains Ranch herd. These cattle were randomly assigned to receive the experimental vaccine (vaccinated group) or a mock vaccine (control group). Randomisation was achieved by assigning a random number to individual cattle ordered by age and breed; cattle assigned an odd number were allocated to the vaccinated group. Before the contact phase of the trial cattle were reassigned new identification numbers to maintain blinding. All research staff involved with the clinical assessment of cattle were blinded to the group allocations.

The animals were checked as being fit and disease-free before the start of the trial. The husbandry was in line with normal on-farm practice. All cattle were vaccinated against foot and mouth disease (Fotivax, Kenya Veterinary Vaccines Production Institute – KEVEVAPI, Nairobi, Kenya) in November 2015 and, during the trial, were sprayed weekly with an acaricide against ectoparasites (DuoDip, Norbrook, Nairobi, Kenya). No other routine vaccinations or treatments were given.

The MCF vaccine virus (Batch Number 2015-01) was produced at Moredun Research Institute (UK) as described previously [15]. Briefly, attenuated AlHV-1 strain C500 was propagated in bovine turbinate cells and cell-free, sterile culture supernatant containing approximately 10^7 TCID50/ml was harvested and stored frozen until required. The vaccinated group cattle received attenuated AlHV-1 C500 virus mixed with the adjuvant Emulsigen® (20% v/v) (MVP, Omaha, USA), whilst the control group cattle received a mock vaccine using Roswell Park Memorial Institute (RPMI) 1640 culture medium (Cedarline Laboratories, Hicksville, USA) and Emulsigen® (20% v/v). Each vaccine dose contained 0.8 ml of virus suspension or culture medium and 0.2 ml of Emulsigen®, inoculated intramuscularly in the neck using a 2 ml syringe and 21 g 1.5 in. needle. The vaccine formulations were administered as a primary inoculation in mid-January (day minus 65) followed by a boost 28 days later in mid-February (day minus 37).

The wildebeest contact phase occurred between mid-March (day 0) and mid-July (day 119) during which time both groups of cattle (vaccinated and control) were grazed as one herd. The livestock herder gently and constantly guided the combined cattle herd to graze near to wildebeest. A 'contact index' was calculated as the product of the amount of time spent grazing with wildebeest (minutes), the number of wildebeest calves present and a numerical score reflecting the distance between the cattle and the wildebeest [7] (Supplementary Material 1). Following the cessation of the contact phase on day 119 when active grazing near to wildebeest was ceased, the cattle were monitored by a livestock herder and research assistant for a further 30 days.

2.4. Clinical assessment

From the start of the wildebeest contact phase, rectal temperatures of the cattle were recorded every second day and every day for cattle with temperatures $\geq 39.0\,°C$. Clinical signs (ocular/nasal discharge, diarrhoea, inappetence) were recorded if present.

2.5. Sampling

Blood was collected from the cattle by jugular venipuncture into 10 ml plain and 10 ml Ethylenediaminetetraacetic acid (EDTA) vacutainer tubes (Becton Dickinson) every 2 weeks beginning on day minus 65 (primary inoculation). Serum was obtained by centrifuging coagulated blood at 3000 rpm for 20 min. Uncoagulated blood was centrifuged at 2000 rpm for 15 min and plasma and buffy coat samples were collected. All samples were stored at $-80\,°C$ before testing.

2.6. Viral DNA in blood

Total DNA was extracted from buffy coat using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) following manufacturer's instructions. Nested PCR amplifying a region of the AlHV-1 gene (ORF 50) was performed as previously described [17]. Briefly, the first round PCR was performed using a minimum of 50 ng of sample DNA with 10 pmol outer forward primer C500-1 and 10 pmol outer reverse primer C500-2, with OneTaq Universal Master Mix (New England Biolabs; containing OneTaq® DNA polymerase, dNTPs, buffer components). Second round amplification was conducted using the first round product (2 μl) with the inner forward primer C500-3 and inner reverse primer C500-4 under the same reaction conditions as the first step. Nested PCR products (10 μl) were analyzed on a 2% (w/v) agarose gel containing 0.4x final concentration GelRed (Biotium, Freemont, USA). Cattle were classified as AlHV-1 positive if one or more PCR analyses were positive.

Three time points 28 days apart were assayed for AlHV-1 DNA during the contact phase of the trial: day 63 (ten days before the first reported death), day 91 and day 119. Terminal blood samples taken before euthanasia for any WA-MCF cases were also assayed.

2.7. Serology

Serum samples were tested for WA-MCF antibodies using an indirect ELISA [15]. Virus-positive and negative ELISA coating antigens were produced from cell-free culture fluid of bovine turbinate (BT) cells infected with attenuated AlHV-1 C500 and from uninfected BT cells respectively [15]. Sera diluted (1:500) in 2% non-fat dried milk/PBS/Tween20 (50 μl) were tested in duplicate as previously described [15]. Optical densities (OD) were read at 450 nm (Synergy HT, Biotek, USA). ELISA values were calculated by subtracting the mean of the negative antigen OD values from the mean of the positive antigen OD values for each sample. Any sample with a negative ELISA value was corrected to zero.

Inter-plate variations were normalised using a correction factor calculated from the mean ELISA values of the positive and negative control samples that were included in all plates as follows.

$$\text{Correction factor} = (P_0 - N_0)/(P_t - N_t)$$

Where: P_0 = Mean of the positive control sera from plate 1
N_0 = Mean of the negative control sera from plate 1
P_t = Mean of the positive control sera from plate on test
N_t = Mean of the negative control sera from plate on test.

The cut-off for assigning a sample as MCF-positive was defined as the mean normalised ELISA values of known negative samples plus three standard deviations, which for this trial was an ELISA value greater than 0.02234.

Six time points were assayed through the trial: (i) day minus 65 (day of prime vaccination); (ii) day minus 23 (two weeks post booster); (iii) day minus 7 (seven days before contact started); (iv) day 63 (ten days before the first death); (v) day 91 and (vi) day 119. Terminal samples collected before euthanasia from any WA-MCF cases were also assayed.

2.8. Pathology

A post mortem examination was carried out on all cattle that succumbed to WA-MCF during which samples were collected in 10% formalin from the lung, liver, kidney, spleen, prescapular and mesenteric lymph nodes and small intestine. Formalin-fixed paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin and evaluated histologically for lesions supportive of WA-MCF infection.

2.9. Case definition

Three case definitions were adopted to classify infection status based on diagnostic test and histopathology results [7].

1. Not infected – AlHV-1 DNA was not detected in buffy coat DNA of cattle in the control group or the vaccinated group and AlHV-1 antibodies were not detected in the control group cattle
2. AlHV-1 infected – AlHV-1 DNA was detected in at least one sample during the trial; and / or AlHV-1 antibodies were detected during the trial (control group cattle only); and the cattle survived.
3. Fatal MCF – AlHV-1 DNA was detected in at least one sample during the trial, the individual died and clinical/post mortem findings (where available) were supportive of WA-MCF.

2.10. Data management and analysis

Data was recorded in Microsoft Excel and statistical analysis was performed in the R statistical software environment (http://CRAN.R-project.org/). Vaccine efficacy and 95% confidence intervals were calculated as described by Hightower et al (1988) [18]. Chi-squared tests were used to compare proportions. A multivariable logistic regression model was developed to examine the outcome related to vaccination accounting for age and breed.

3. Results

3.1. Vaccine safety

Four cattle displayed symptoms suggestive of a mild adverse reaction within 30 min of the primary inoculation being administered. Three of these cattle were in the control group and one in the vaccinated group. Reactions included depression (3/4), facial

swelling (2/4), recumbency (2/4). One animal (control group) had salivation and increased respiratory effort. This animal was treated with 20 mg dexamethasone (Dexajet, Dawa Ltd, Kenya) administered intramuscularly. The vaccinated animal was subdued, had facial swelling and lay in sternal recumbency. All affected cattle recovered within 24 h. There were no recorded adverse reactions following administration of the booster inoculation.

3.2. Wildebeest contact

From day 28 after the booster inoculation (day zero of contact), the cattle were grazed with wildebeest. The change in contact index during the trial period is represented in Supplementary Material 1 and Supplementary Fig. 1.

3.3. Clinical assessment

The trial outcome for each individual is shown in Supplementary Material 2. Two cattle, one in each group, died before the start of the wildebeest contact phase of the trial. Because the pathology suggested that the cause of death was unrelated to WA-MCF, these cattle were excluded from the analysis (Supplementary Material 3). A vaccinated animal was lost to predation during the contact phase of the trial and has also been excluded from the analysis.

Twenty-five cattle showed clinical signs suggestive of WA-MCF. Detected signs included nasal and ocular discharge, inappetence, depression, and pyrexia. Twenty-two of these affected cattle were euthanized on humane grounds as they reached a clinical score of 6, one animal died suddenly and two were lost to predation on days 108 and 120 post contact with wildebeest. Since both of the lost cattle exhibited clinical signs of WA-MCF, they were not excluded from the analysis (number of cattle that were susceptible to disease). No post-mortem samples were obtained from the predated cattle.

Four cattle developed pyrexia during the wildebeest contact phase of the trial with unspecified conditions, the clinical presentation of which (described in full in Supplementary Material 3) did not resemble that of WA-MCF. Consequently, these cattle were not considered cases.

3.4. WA-MCF due to AlHV-1 infection

The first case of WA-MCF was detected 71 days after the start of the wildebeest contact phase. Twenty-five cattle succumbed to WA-MCF between days 73 and 130 with the onset of clinical signs peaking between days 92–112 post contact and the majority of deaths between days 99–112 (Fig. 1). Two cattle from the control group predated on day 108 and 121 respectively were included in the analysis since they were assessed as having clear clinical signs of WA-MCF and were AlHV-1 PCR positive in samples taken during the week before predation. Four of 71 (5.6%) cattle in the vaccinated group and 21 of 72 (29.2%) control group cattle succumbed to WA-MCF. There was a significant difference in WA-MCF between the vaccinated and control groups (OR = 7.1, 95% CI = 2.5–25.5) (Table 1). However, breed (OR 1.4; 95% CI 0.52–3.61) and age (OR 1.00; 95% CI 0.99–1.01) were not significant determinants of WA-MCF in the logistic regression model (Table 1).

3.5. PCR

Viral DNA was detected in 20 (28.8%) vaccinated group cattle and 35 (48.8%) control group cattle during the challenge phase of the trial (χ^2 = 5.98, df = 1, p = 0.01). The individual results for all cattle are presented in Supplementary Material 4.

The number of cattle in each group presenting with new AlHV-1 infections at each time point is shown in Fig. 2. Nine cattle (5 in the vaccinated group and 4 in the control group) maintained infection over two time points and 12 cattle (6 from the vaccinated group and 6 from the control group) resolved infections between time points. Regarding the cattle that died, AlHV-1 DNA was detected in all terminal samples and also at one sampling time point before the onset of clinical signs in 12/21 control group cattle and 2/4 vaccinated group cattle.

3.6. Serological response

Nine (6.3%) cattle (4 control group and 5 vaccinated group) had positive serological responses before the trial. At the next time point (two weeks after boost) the antibody levels for the control
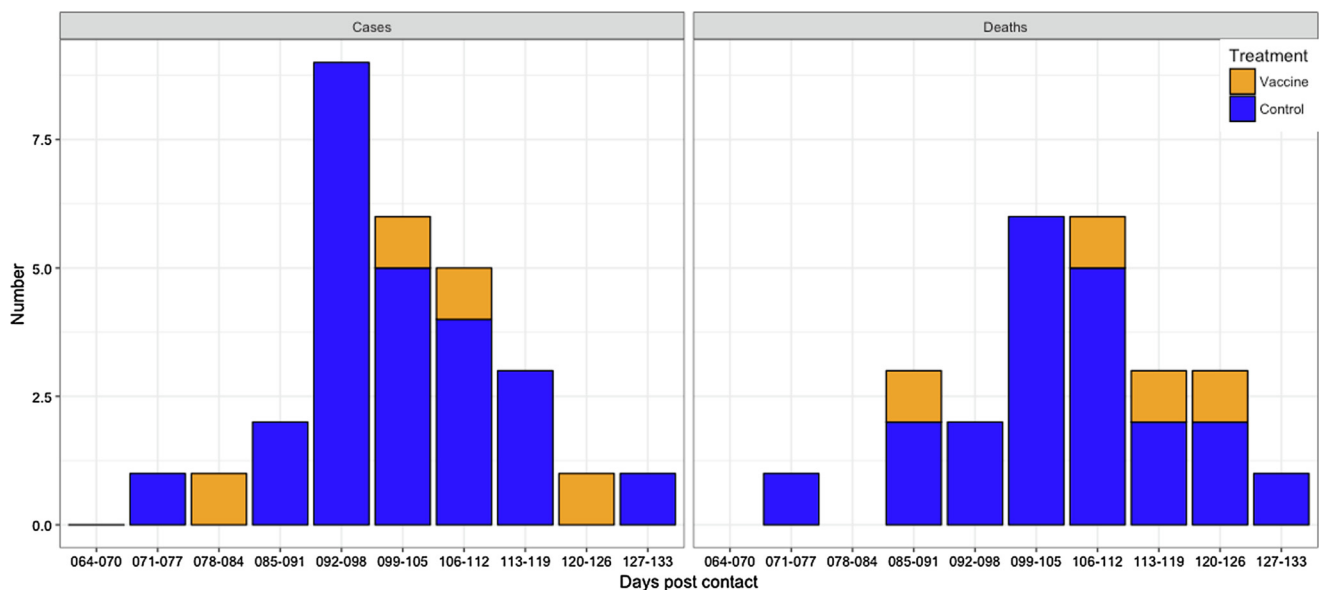

Fig. 1. Epidemic curves indicating the onset of clinical signs (cases) and deaths of WA-MCF affected cattle in 7-day intervals. Orange squares represent cattle in the vaccinated group and blue squares represent cattle in the control group.

Table 1
Results of the multivariable logistic regression analysis comparing mortality between cattle in the vaccinated and control groups adjusting for age and breed.

	WA-MCF % (95% CI)	OR (95% CI)	P value
Boran	16/98 16.3% (10.3–24.8)	1	
BoranX Friesian	9/45 20.0% (11.0–33.7)	1.4 (0.52–3.61)	0.501
Age	–	1.00 (0.99–1.01)	0.754
Vaccinated	4/71 5.6% (2.3–13.6)	1.00	
Control	21/72 29.2% (19.7–40.7)	7.1 (2.5–25.5)	<0.001

group cattle with positive serological responses prior to vaccination fell below the cut-off. Two of the 4 control group cattle with positive serological responses prior to vaccination died of WA-MCF. None of the vaccinated group cattle with positive serological responses prior to vaccination died of WA-MCF.

All vaccinated group cattle mounted a serological response following vaccination (Supplementary Material 5). Peak antibody levels were detected two weeks after boost (Fig. 3). Two of the 4 vaccinated group cattle that died of WA-MCF showed an increasing antibody response in terminal samples.

Twenty-seven (37.8%) control group cattle demonstrated a serological response in at least one sample collected during the contact phase (Fig. 3). A number of seropositive control group cattle developed an antibody response greater than the median of the vaccinated group cattle during the later stages of the trial. Only 5 control group cattle maintained positive serological responses over two time points; 4 of these died of WA-MCF (Fig. 4).

3.7. Histopathology

Histologically the changes in all cattle were supportive of a diagnosis of MCF. The severity of changes varied both between tissues and between animals with the most consistent lesions being lymphoid hyperplasia in the lymph nodes and spleen, interstitial pneumonia and nephritis and portal hepatitis. Vascular damage of varying degrees was present in all tissues evaluated but arteritis was not detectable in all tissues. Where present, the features of arteritis included lymphocytic cellular infiltrates in the tunica adventitia and/or media of medium to larger caliber arteries and

occasionally fibrinoid degeneration of the tunica media. In many tissues the pattern of vascular damage was a more subtle swelling or vacuolation of endothelial cells. Other significant histological changes include urothelial erosion in all cases where urothelium was represented and alveolar hyaline membrane formation in the lungs of occasional cases.

3.8. Vaccine efficacy and case definitions

Of the cattle in the control group, 45 (63%) showed evidence of exposure to WA-MCF either by seroconversion (n = 10), viral DNA by PCR (n = 18) or both (n = 17). The fatality rate (21/45) of control group cattle that showed evidence of exposure was 46.7% (95% CI 33.2–60.9%). The cattle in the control group that died of MCF (n = 21) were all AlHV-1 positive by PCR. Of the cattle in the vaccinated group, 20 (28%) showed evidence of exposure to WA-MCF through detection of viral DNA by PCR. The fatality rate (4/20) of vaccinated group cattle that showed evidence of exposure was 20.0% (95% CI 8.2–42.1%).

The vaccine efficacy at preventing infection with AlHV-1 detected by PCR alone was 42.1% (95% CI 9.9–62.7%). The vaccine efficacy at preventing infection, taking into account both serological and PCR evidence, was 54.9% (95% CI% 31.9–70.2%).

Of the 25 cattle that showed clinical signs suggestive of WA-MCF, 23 met the case definition for fatal MCF, 19 in the control group and 4 in the vaccinated group (Table 2). The vaccine efficacy at preventing mortality was 79% (95% CI 42.1–92.5%). When the 2 predated WA-MCF-affected cattle were included in the analysis the vaccine efficacy was 80.6% (95% CI 46.5–93.0%).

There were 9 seropositive cattle before the start of the trial (5 cattle in the vaccinated group and 4 in the control group). Since it is not known if prior exposure to AlHV-1 in cattle is protective the vaccine efficacy calculation was repeated excluding these cattle. The case fatality rate in the vaccinated group cattle was 4/66 (6%) and in the control group cattle 19/68 (28%). The vaccine efficacy for preventing mortality with these exclusions was 78.3% (95% CI 39.6–92.2%).

4. Discussion

In this study we have shown for the first time that the attenuated AlHV-1 C500 vaccine protects cattle grazed with wildebeest under field conditions against fatal WA-MCF with mortality due to WA-MCF in vaccinated cattle being significantly lower than in
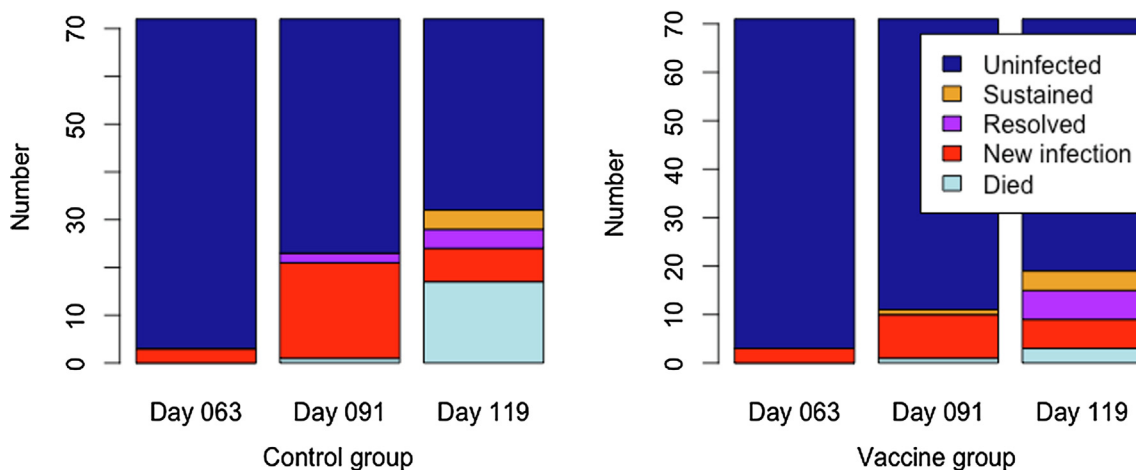

Fig. 2. The number of cattle that were AlHV-1 infected (DNA positive) between days 63 and 119. The histograms are divided into the control group on the left and vaccinated group on the right. Cattle are colour coded according to their infection status at each time point. Dark blue – uninfected, orange – sustained infection, purple – resolved infection, red- new infections, light blue – dead.
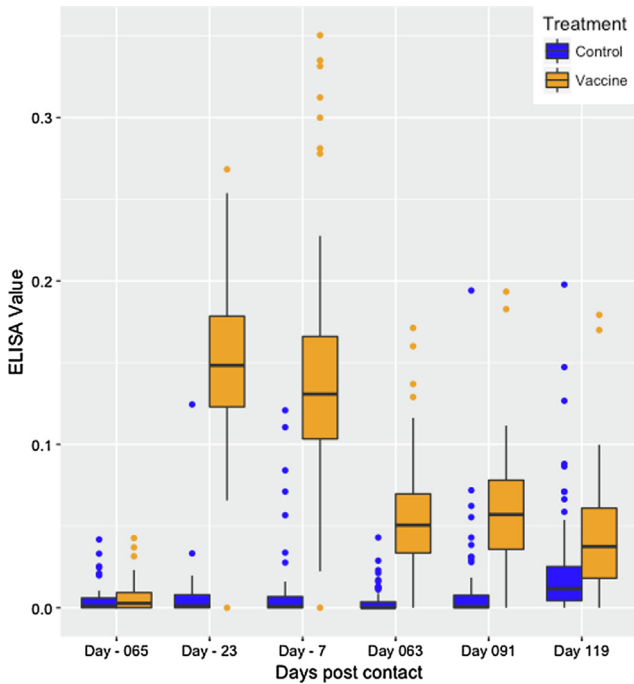
Fig. 3. Boxplot demonstrating the serological response in vaccinated cattle (orange) and control cattle (blue) over the course of the field trial.
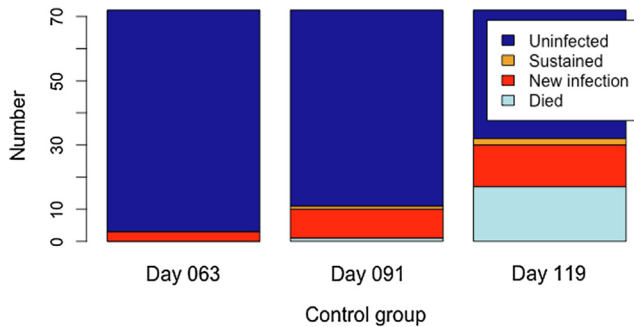

Fig. 4. The number of control group cattle that were ELISA positive between days 63 and 119. Cattle are colour coded according to their infection status at each time point. Dark blue – uninfected, orange – sustained infection, red- new infections, light blue – died.

control cattle. This was not demonstrated in the previous field experiment [7]. In addition, vaccination protected cattle against infection, with the detection of AlHV-1 DNA in vaccinated cattle being just over half that of the control group. This is consistent

with the findings of the previous field trial in Tanzania [7]. A vaccine with an efficacy approaching 80% would likely be popular amongst cattle keeping people whose herds are at risk of fatal WA-MCF and who currently have to employ costly annual disease avoidance strategies. Strategies that, although largely WA-MCF sparing, negatively impact not only the health and condition of their cattle but also their livelihoods, principally through restrictions on their ability to sell milk [13]. Given many of the at-risk cattle owners are politically and economically marginalised [19], an effective vaccine that reduces the burden of this disease could have enormous merit.

Mortality from WA-MCF is generally reported to be 100% [8], however this calculation is based on survival of clinically-affected cattle. In this study, mortality among cattle in the control group with evidence of AlHV-1 infection was 47%. The cattle in the control group that were AlHV-1 DNA positive (n = 14) or that seroconverted during the trial (n = 10) and did not succumb to infection (n = 24) indicated that sub-clinical WA-MCF infections do occur in cattle, as suggested previously [7,20–23]. Factors that determine the outcome of such infections might include infectious dose and environmental and host factors such as genetic susceptibility/resistance, as has been previously described in bison (*Bison bison*) [21,24]. Additionally it has been hypothesised that cattle in East Africa may have developed resistance to endemic pathogens including AlHV-1 through repeated exposure [25].

The causative agents of MCF comprises a number of gammaherpesviruses including AlHV-1, alcelaphine herpesvirus 2 (AlHV-2), caprine herpesvirus 2 (CpHV-2), ibex MCF (MCFV-ibex), MCFV in white-tailed deer (MCFV-WTD), and ovine herpesvirus 2 (OvHV-2) [26]. At the study site, Kapiti Plains Ranch, there are sheep and goats the natural reservoirs of OvHV-2 as well as wildebeest. All of the MCF cases identified in this work occurred within the context of a specific time window where cattle were deliberately exposed to close contact with calving wildebeest rather than resident goats and sheep. These cases were analysed by AlHV-1 specific nested PCR and all of the clinical WA-MCF cases were found to contain AlHV-1 DNA in tissue samples. It remains possible that the nine animals that had positive serological responses at the start of the trial could have been infected by an MCF virus other than AlHV-1. However, the serological testing available to us could not distinguish between the different MCF viruses. Cross reactive serology is an issue where all MCF serological tests (ELISA, cELISA) use antigen from AlHV-1 [26]. Some assays can be used to distinguish infection with different MCFV, such as cross-neutralisation experiments using reservoir species serum [27] but these cannot be applied to cattle serum. The potential for cross protection using the AlHV-1 C500 vaccine for other MCFV is unknown. Taus et al. demonstrated that neutralising antibodies are host and pathogen specific with AlHV-1 neutralising antibodies detected in wildebeest but not goats and OvHV-2 neutralising antibodies detected in goats but not wildebeest [27]. The lack of cross-neutralisation may indicate the extent of expected cross protection.

Table 2
Case definitions of cattle during the contact phase of the WA-MCF vaccine field trial.

Group	Died	WA-MCF Histo-pathology	PCR positive	ELISA positive[†]	ELISA and PCR positive	Case definition[‡]
Vaccinated n = 71	4	4	20	NA	NA	(1) 51
						(2) 16
						(3) 4
Control n = 72	21	19	35	27	45	(1) 27
						(2) 24
						(3) 21*

[*] Including the two predated cattle, which could not be assessed by histopathology.
[†] ELISA positive is defined as at least one positive test (control cattle); this could not be calculated for cattle in the vaccine group.
[‡] (1) Not infected; (2) AlHV-1 infected; (3) Fatal MCF.

The vaccine induced a detectable serological response in all cattle in the vaccine group, peaking six weeks after the prime vaccination (Fig. 3). The cattle in the vaccinated group that died of WA-MCF (n = 4), showed an increased antibody response associated with the development of clinical WA-MCF. The increase in virus specific antibody in cattle in the vaccinated group has previously been reported in cattle that succumbed to clinical WA-MCF [15,28]. A serological response to WA-MCF was also detected in ten of the cattle in the control group that died during the contact phase. In previous laboratory challenge experiments cattle in the control group did not demonstrate a significant serological response following infection [15]. Contrasting conditions between laboratory experiments (in which cattle receive only one intranasal challenge dose) and field trials could explain the different serological patterns. Cattle in field conditions are likely to receive multiple small exposures over time simulating the development of virus specific antibodies.

It does not appear that virus specific antibody responses in the control group were protective against fatal WA-MCF. Two of the cattle in the control group that were seropositive at the beginning of the trial later died of WA-MCF, suggesting that subclinical exposures are not protective. Additionally, subclinical exposure to AlHV-1 in the vaccinated group did not appear to prevent the vaccine from giving protection since there were no WA-MCF cases in the cattle in the vaccine group that were seropositive before the prime dose was administered. There is currently no evidence to suggest that subclinical WA-MCF infection can lead to later disease, nor that field exposure of sub-clinically infected cattle leads to WA-MCF due to reactivation of latent virus. Further research is required to understand the relationship between subclinical exposure and subsequent WA-MCF disease outcomes. Since it is not known if early field challenge may interfere with development of protection from the vaccine, the seasonal nature of WA-MCF suggests that vaccination in the face of disease pressure can be avoided.

The nine (6%) cattle that were seropositive for AlHV-1 antibodies at the start of the trial indicate prior exposure to AlHV-1, possibly during the 2015 WA-MCF epidemic at Kapiti Plains Ranch, which clinically affected 4% of cattle, and also provides further evidence that subclinical MCF virus infections occur [10]. It has been reported that the serological response to vaccination wanes within 6 months but there is no data regarding the longevity of antibody responses from subclinical exposure [15]. Previous research reported the seroprevalence of AlHV-1 antibodies in cattle in endemic areas of Tanzania to be 1% [7]. The observed difference in seroprevalence between these two endemic areas may be the result of different husbandry and WA-MCF avoidance strategies employed by the Maasai pastoralists in Tanzania [13] and of the Kapiti Plains Ranch where movement away from wildebeest is restricted by property boundaries. The effectiveness of traditional disease avoidance strategies at reducing AlHV-1 infection is further supported by the difference in the proportions of fatal WA-MCF among the cattle in the control group (29%) compared with that reported in other studies in East Africa (3–12%) [8]. The increased prevalence of MCF in this trial is almost certainly the result of actively herding cattle towards groups of wildebeest during the challenge phase of the trial. This approach was successfully used in previous trials in Tanzania [7]. In the absence of such behaviour however, MCF rates on the Kapiti Plains Ranch varied from 1.5% to almost 9% between 2014 and 2016 [10], suggesting that a range of factors influence prevalence of MCF.

Six of the cattle in the control group were AlHV-1 positive in only a single time point sample. It is not possible to determine if these cattle cleared an acute subclinical infection or became latent carriers. Defining whether these subclinically infected cattle develop lifelong infection with the potential for recrudescence is of interest both with respect to our understanding of MCF and for broader herpesvirus biology [21,22].

The primary inoculation resulted in a small number of cattle having moderate reactions, although the majority of these were in control group cattle. Emulsigen® is an oil-in-water adjuvant with no known toxicities. The decision to include Emulsigen in the vaccine preparation was based on previous (unpublished) work that demonstrated inoculation of cattle with attenuated AlHV-1 in the absence of adjuvant did not protect from WA-MCF (Russell & Benavides, unpublished data). A small number of adjuvants (both conventional and mucosal) were previously tested in comparison with the original Freunds formulation, but Emulsigen was found to be both efficacious and induced minimal injection site reactions ([5,15,25]; Russell unpublished). The vaccine in combination with Emulsigen appears to be safe as demonstrated in previous trials [7]. However, cattle keepers may not be equipped to respond to vaccine reactions in an appropriate or timely manner and the management of these cases needs to be considered in future. Further monitoring of the safety of the vaccine will be carried out over subsequent trials and further experiments trialling other adjuvants may be required.

The distribution of WA-MCF is geographically limited to the range of wildebeest in eastern and southern Africa [12]. The introduction of a protective vaccine for MCF may change the behaviour of cattle owners in these areas. For example, where cattle owners traditionally avoid wildebeest, the use of an effective vaccine might lead to increased encroachment of protected lands and overgrazing in wildlife dispersal areas [7]. On the other hand, changes in land use patterns and increased fragmentation (and fencing) of previously communal lands have made traditional WA-MCF avoidance strategies increasingly difficult to employ [7]. In situations such as this, where cattle cannot be herded away from wildebeest and their young calves, an effective vaccine would have merit. Consequently, both the potential market for the AlHV-1 C500 vaccine and the environmental impacts need to be assessed.

5. Conclusions

This trial demonstrated the efficacy of the attenuated AlHV-1 vaccine to reduce mortality from WA-MCF in cattle and suggests that this vaccine could have an important role to play in protecting cattle from this lethal disease. The trial also raised questions regarding the role of sub-clinical disease in the epidemiology of WA-MCF. It is likely that the true number of infected cattle is generally underestimated. Cattle are considered dead-end hosts so subclinical cases are unlikely to play a role in the transmission of AlHV-1. However, the potential for recrudescence or chronic disease may increase the impact of WA-MCF on cattle owners in the region beyond the death of acute cases. There is little evidence from this study to demonstrate that prior exposure is protective against fatal infection, but further investigation is required to confirm this and establish any long-term risks posed by sub-clinical MCF infection. The establishment of non-fatal infections may also suggest the potential for genetic resistance and further investigation in endemic areas where selection pressure is high should be considered.

Animal welfare

The ILRI Institution Animal Care and Use Committee approved the study (IACUC 2016-01). The Directorate of Veterinary Services, Republic of Kenya granted approval for the trial (VACC/I/VOL. XV/79).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of materials

All the relevant data for each individual is valuable in Supplementary Table 2.

Author contributions

EAJC contributed to the design, data collection, analysis and prepared the manuscript. DG contributed to the conceptualisation and reviewed the manuscript. CM contributed to sample analysis and interpretation and reviewed the manuscript. LO contributed to the clinical assessment of cattle, data collection and reviewed the manuscript. ED contributed to histopathological examination and interpretation and reviewed the manuscript. GR, FL and VN contributed to the design, analysis and critically reviewed the manuscript. All authors read and approved the final manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.08.040.

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