Safety and immunogenicity of a ChAdOx1 vaccine against Rift Valley fever in UK adults: an open-label, non-randomised, first-in-human phase 1 clinical trial

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For the Swahili translation of the abstract see Online for appendix 1

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Summary

Background Rift Valley fever is a viral epidemic illness prevalent in Africa that can be fatal or result in debilitating sequelae in humans. No vaccines are available for human use. We aimed to evaluate the safety and immunogenicity of a non-replicating simian adenovirus-vectored Rift Valley fever (ChAdOx1 RVF) vaccine in humans.

Methods We conducted a phase 1, first-in-human, open-label, dose-escalation trial in healthy adults aged 18–50 years at the Centre for Clinical Vaccinology and Tropical Medicine, Oxford, UK. Participants were required to have no serious comorbidities or previous history of receiving an adenovirus-based vaccine before enrolment. Participants were non-randomly allocated to receive a single ChAdOx1 RVF dose of either 5×10^9 virus particles (vp), $2{\cdot}5\times10^{10}$ vp, or 5×10^{10} vp administered intramuscularly into the deltoid of their non-dominant arm; enrolment was sequential and administration was staggered to allow for safety to be assessed before progression to the next dose. Primary outcome measures were assessment of adverse events and secondary outcome measures were Rift Valley fever neutralising antibody titres, Rift Valley fever GnGc-binding antibody titres (ELISA), and cellular response (ELISpot), analysed in all participants who received a vaccine. This trial is registered with ClinicalTrials.gov (NCT04754776).

Findings Between June 11, 2021, and Jan 13, 2022, 15 volunteers received a single dose of either 5×10^9 vp (n=3), $2{\cdot}5\times10^{10}$ vp (n=6), or 5×10^{10} vp (n=6) ChAdOx1 RVF. Nine participants were female and six were male. 14 (93%) of 15 participants reported solicited local adverse reactions; injection-site pain was the most frequent (13 [87%] of 15). Ten (67%) of 15 participants (from the $2{\cdot}5\times10^{10}$ vp and 5×10^{10} vp groups only) reported systemic symptoms, which were mostly mild in intensity, the most common being headache (nine [60%] of 15) and fatigue (seven [47%]). All unsolicited adverse events reported within 28 days were either mild or moderate in severity; gastrointestinal symptoms were the most common reaction (at least possibly related to vaccination), occurring in four (27%) of 15 participants. Transient decreases in total white cell, lymphocyte, or neutrophil counts occurred at day 2 in some participants in the intermediate-dose and high-dose groups. Lymphopenia graded as severe occurred in two participants in the 5×10^{10} vp group at a single timepoint, but resolved at the subsequent follow-up visit. No serious adverse events occurred. Rift Valley fever neutralising antibodies were detectable across all dose groups, with all participants in the 5×10^{10} vp dose group having high neutralising antibody titres that peaked at day 28 after vaccination and persisted through the 3-month follow-up. High titres of binding IgG targeting Gc glycoprotein were detected whereas those targeting Gn were comparatively low. IFNγ cellular responses against Rift Valley fever Gn and Gc glycoproteins were observed in all participants except one in the 5×10^{10} vp dose group. These IFNγ responses peaked at 2 weeks after vaccination, were highest in the 5×10^{10} vp dose group, and tended to be more frequent against the Gn glycoprotein.

Interpretation ChAdOx1 RVF was safe, well tolerated, and immunogenic when administered as a single dose in this study population. The data support further clinical development of ChAdOx1 RVF for human use.

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Introduction

Rift Valley fever is a mosquito-borne viral zoonosis that primarily affects domestic livestock (eg, sheep, goats, and cattle) and humans in Africa and the Middle East.[1] The disease was first identified in Kenya in 1930 and is characterised by high rates of death (>90%) in young animals and pregnancy loss (typically >90%) in animals that are pregnant.[2] Spillover into human populations has primarily been attributed to direct contact with infected animal tissues, although mosquito transmission does occur.[2] Human disease can vary widely; although most people will have a self-limiting febrile illness, an estimated 0·5–3% develop severe symptoms, such as haemorrhagic diatheses, in which case fatality can be as

Research in context

Evidence before this study

Both WHO and the African Union have identified vaccine development for Rift Valley fever in humans as an urgent priority because of the potential of Rift Valley fever to cause a public health emergency with severe health consequences and major economic impacts. The aim of Rift Valley fever vaccinology has been to design vaccines that are safe and highly immunogenic for neutralising antibodies, which are associated with protection, as well as having other optimal product characteristics defined by WHO. We conducted a search for Rift Valley fever vaccine trials on ClinicalTrials.gov using the search terms "Rift Valley Fever" AND "vaccine" with no date or language restrictions. Two candidate vaccines, the inactivated TSI-GSD-200 vaccine and the live-attenuated MP-12 vaccine, have previously been evaluated in humans and been shown to safely elicit neutralising antibodies. TSI-GSD-200 has been registered on ClinicalTrials.gov as currently recruiting for a phase 2 trial, but there have been no further updates on the clinical development of MP-12 for human use since completion of a phase 2 trial in 2009. The non-replicating simian adenovirus-vectored Rift Valley fever vaccine (ChAdOx1 RVF), described in this Article, is also listed as undergoing a separate phase 1b trial in Uganda (NCT04672824). As with TSI-GSD-200 and MP-12, further evaluation of ChAdOx1 RVF in humans comes after safety, immunogenicity, and efficacy against Rift Valley fever has been shown in livestock.

Added value of this study

This Article describes a first-in-human trial of ChAdOx1 RVF, a chimpanzee adenovirus-vectored Rift Valley fever vaccine that has been shown to be highly immunogenic and efficacious against Rift Valley fever in all major livestock species affected by the disease. Although the ChAdOx1 platform is deployed for use against COVID-19 (eg, the Oxford and AstraZeneca AZD1222 vaccine) in more than 180 countries, this study is the first clinical evaluation of its use for Rift Valley fever in humans. We assessed the safety and immunogenicity of a single intramuscular dose of ChAdOx1 RVF among adults in the UK; it was well tolerated with no serious adverse events. High Rift Valley fever neutralising-antibody titres were detected within 2 weeks of vaccination, peaking at 28 days after vaccination. A strong Rift Valley fever viral glycoprotein-specific IFNγ response, peaking at 2 weeks after vaccination, was also detected. Both humoral and cellular responses persisted during the 3-month follow-up period of the study.

Implications of all the available evidence

A vaccine for use against Rift Valley fever in humans remains an urgent unmet need. ChAdOx1 has been shown to be a scalable vaccine platform for COVID-19, but this is the first use of the platform for Rift Valley fever in humans. ChAdOx1 RVF was well tolerated and generated strong humoral and cellular immune responses. Further evaluation of the vaccine in populations at most risk for Rift Valley fever is warranted.

high as 50%.[3] Other severe complications of Rift Valley fever, such as meningoencephalitis and ocular pathology, can lead to debilitating sequelae (eg, blindness),[3] and infection during pregnancy carries an increased risk of miscarriage.[4] Licensed vaccines are available for livestock,[5] but no licensed Rift Valley fever vaccines are available for human use. Both WHO and the African Union have prioritised Rift Valley fever for urgent development of vaccines and other countermeasures.[6]

Natural exposure to the Rift Valley fever virus generates long-lived protective neutralising antibodies in both humans and livestock.[7,8] When passively transferred into mice, human serum samples containing neutralising antibodies confer protection against Rift Valley fever viral challenge in a dose-dependent manner, supporting the importance of neutralising antibodies in protection.[9] These neutralising antibodies target the Rift Valley fever viral envelope glycoproteins, Gn and Gc, that are well conserved across virus strains and therefore provide cross-protective immunity against virus lineages from distant geographical settings.[10] The aim of Rift Valley fever vaccinology has therefore been to design vaccines that are safe and highly immunogenic for protective neutralising antibodies, in addition to meeting other optimal product characteristics defined by WHO, such as the ability to be administered in a single dose, maintenance of immunity for at least 1 year, and long-term product stability.[6] The main target population for a human Rift Valley fever vaccine are people who live in areas prone to outbreaks, especially those in contact with livestock including herders, farmers, abattoir workers, and veterinarians.[6] Typically, Rift Valley fever outbreaks in livestock tend to precede outbreaks in humans, emphasising the importance of livestock vaccination to not only minimise animal losses, but also limit virus transmission to humans.[11] However, livestock vaccination does not obviate the need for a human vaccine as routine livestock vaccination will rarely approach 100% compliance, human cases have been reported in the absence of livestock outbreaks, and there is a potential for epidemic spread.[12]

Two Rift Valley fever vaccine candidates have previously been evaluated in humans. The first is an inactivated vaccine, TSI-GSD-200, which had a good safety profile but, even after an initial three-dose regimen, approximately 10% of vaccinated participants did not have seroconversion.[13] The second is a live-attenuated vaccine, RVF MP-12. This vaccine was investigated in

small clinical trials showing a favourable safety and immunogenicity profile in humans,[14] but further updates on its development have mainly been for its use as a veterinary vaccine.[15] Since 2012, a One Health approach to Rift Valley fever vaccinology has been taken by developing a single vaccine, ChAdOx1 RVF, for use in both humans and livestock. The vaccine, composed of the ChAdOx1 adenovirus vector expressing a codon-optimised transgene for the Rift Valley fever (RVF) viral Gn and Gc glycoproteins (GenBank accession number DQ380208),[16] uses the same ChAdOx1 adenovirus vector platform used to make the ChAdOx1 nCoV-19 COVID-19 vaccine that has been deployed in more than 180 countries,[17] including in Africa, where the current burden of Rift Valley fever is.[1]

See Online for appendix 2

ChAdOx1 RVF has shown remarkable safety, immunogenicity, and 100% efficacy against wild-type Rift Valley fever virus challenge in sheep, goats, and cattle in Kenya.[16] More recently, it was found to safely provide protection against disease and fetal loss in pregnant sheep and goats,[18] supporting its further development for veterinary use. In this study we aimed to evaluate the safety, tolerability, and immunogenicity of ChAdOx1 RVF vaccine in a first-in-human phase 1 trial of healthy adults in the UK.

Methods

Study design and participants

We conducted a phase 1, first-in-human, dose-escalation, open-label, non-randomised clinical trial of the ChAdOx1 RVF vaccine at the Centre for Clinical Vaccinology and Tropical Medicine, Oxford, UK. Healthy adult volunteers aged 18–50 years were recruited from the local Oxfordshire area with ethically approved advertising materials, such as posters, the recruiting trials website of the study site, and social media. All advertising text and materials were approved by the National Health Service (NHS) East of England–Cambridge East Research Ethics Committee (20/EE/0262). Potential volunteers initially completed an online questionnaire covering major exclusion criteria. They were then invited for an in-person screening visit where written informed consent for the study was obtained followed by a medical history assessment, physical examination, urine analysis, and clinical blood tests. Medical histories were corroborated with medical records obtained from the general practitioner (GP) of each volunteer before enrolment. Volunteers who had previously been vaccinated with a ChAdOx1 vaccine (eg, ChAdOx1 nCoV-19) were excluded from the study. Additionally, volunteers with a history of travel to countries endemic for Rift Valley fever were screened with a commercial Rift Valley fever ELISA (ID Screen Multispecies RVFV ELISA, ID.vet [Innovative Diagnostics, Montpellier, France]) as per manufacturer's instructions and excluded if seropositive. Participants were asked to self-report their sex as female or male. Results of all screening assessments were reviewed by a trial investigator before enrolment or exclusion. Full eligibility criteria for the trial are detailed in the trial protocol (appendix 2 p 43).

The trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. Regulatory approval was granted by the UK Medicines and Healthcare Products Regulatory Agency (CTA 21584/0438/001–0001) and ethics approval by NHS East of England–Cambridge East Research Ethics Committee. Use of ChAdOx1 RVF for this clinical trial was authorised by the Oxford University Hospital NHS Trust Genetic Modification Safety Committee (GM462.18.103).

Procedures

The ChAdOx1 RVF vaccine (formerly known as ChAdOx1 GnGc) has been described previously and was manufactured by Advent (Rome, Italy) in accordance with current Good Manufacturing Practices as described in the investigational medicinal product dossier.[16] All participants received a single dose of ChAdOx1 RVF, administered intramuscularly into the deltoid of their non-dominant arm. Participants were sequentially (non-randomly) allocated to one of three escalating dose groups, starting with an initial low-dose group (5×10^9 virus particles [vp] of ChAdOx1 RVF, n=3), before progression to an intermediate-dose group ($2\cdot5\times10^{10}$ vp, n=6), and finally a high-dose group (5×10^{10} vp, n=6). Investigators and participants were not masked; allocation was done by investigators. A local safety monitor was appointed to provide safety oversight for the trial who conducted the interim safety reviews before each dose escalation containing a minimum of 7 days safety data from all participants of the preceding group. Enrolment was also staggered within groups, with the first participant in each group being vaccinated alone followed by a review 48 h before enrolment of the next two participants. A further minimum interval of 48 h was observed before the vaccination of the final three participants in the intermediate-dose and high-dose groups.

After vaccination, participants attended a series of follow-up visits at the following nominal timepoints: day 2, 7, 14, 28, 56, and 84. Participants also completed a daily online symptom diary for 28 days after vaccination. Solicited adverse events were monitored for 7 days and unsolicited adverse events (all other events not defined as solicited) for 28 days after vaccination. Occurrence of serious adverse events was assessed at all follow-up visits. Clinical laboratory blood tests, including full blood count, liver function, renal function, and electrolytes, were done at day 0 (immediately before vaccination), day 2, day 7, and day 28. Laboratory adverse events were graded by use of toxicity tables, which were adapted from the US Food and Drug Administration toxicity-grading scale. Unsolicited adverse events were coded with the Medical Dictionary for Regulatory Activities version 24.0

and assessed by investigators for causality with ChAdOx1 RVF. Blood samples for immunology assays were taken on day 0 and at days 7, 14, 28, 56, and 84. The schedule of timepoints for all immunogenicity measures was specified in a laboratory analysis plan before the enrolment of the first participant.

Electronic data capture and clinical data management were done with OpenClinica open-source software, version 4.0.

Outcomes

The primary objective of the study was assessment of safety and tolerability of the ChAdOx1 RVF vaccine in a healthy adult population. Primary outcome measures were occurrence of local and systemic solicited adverse events for 7 days after vaccination, occurrence of unsolicited adverse events for 28 days after vaccination, changes in clinical laboratory measures from baseline to day 28, and occurrence of serious adverse events throughout the trial period. The secondary objective was humoral and cellular immunogenicity of the vaccine. Secondary outcome measures were Rift Valley fever neutralising antibody titres measured against live Rift Valley fever virus, IgG binding antibody titres measured by ELISA against recombinant Gn and Gc proteins, and cellular responses to overlapping peptides spanning the Gn–Gc polyprotein measured by ex-vivo IFNγ enzyme-linked immunospot (ELISpot) assay. Exploratory outcomes measures were IgG1–4 subclass antibody ELISA titres against Gn and Gc proteins and analysis of correlations between immune variables. Full details of the immunological assay procedures are provided in appendix 2 (p 2).

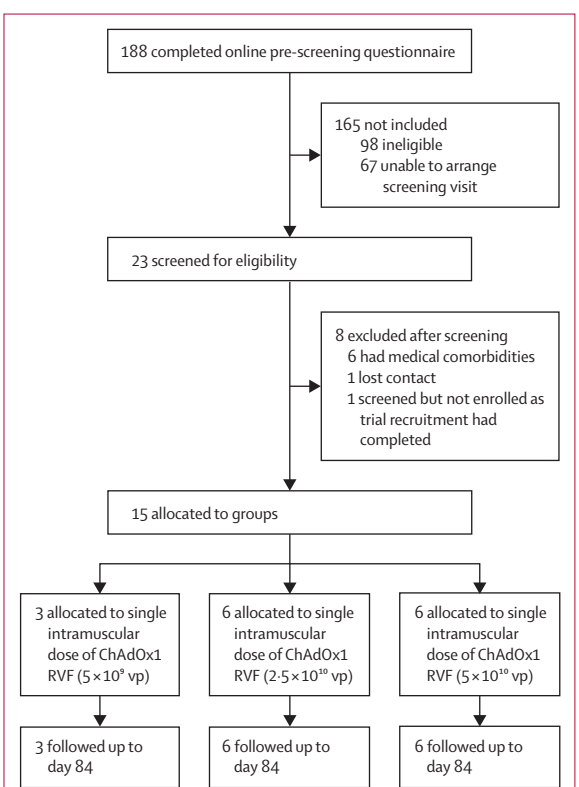

Figure 1: **Trial profile**
RVF=Rift Valley fever. vp=virus particles.

Statistical analysis

This phase 1, first-in-human trial aimed to describe the safety, tolerability, and immunogenicity of ChAdOx1 RVF. The number of participants in each vaccine dose group allowed a descriptive analysis of the frequency and magnitude of adverse events after vaccination, rather than statistical significance testing for safety differences between individuals. Descriptive statistics were compiled in Microsoft Excel version 2108. We planned to include all participants who received a vaccine in all analyses. Immunological data were visualised and analysed with non-parametric tests on GraphPad Prism version 9 (GraphPad Software, Boston, MA, USA), with a two-sided α of 0·05 for statistical significance. All analyses of correlations were done with non-parametric Spearman's correlation tests. This trial is registered with ClinicalTrials.gov, number NCT04754776. There was no data monitoring committee for this trial.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report other than reviewing the proposed study design during the funding application.

Results

Between June 2, 2021, and Jan 7, 2022, 188 potential volunteers completed our online pre-screening questionnaire, most of whom (165 [88%]) were ineligible or unable to arrange a screening visit. The remaining 23 volunteers were screened for eligibility, of whom 15 eligible volunteers with a median age of 25 years (range 20–38) were enrolled into the study between

	5×10^9 vp (n=3)	$2{\cdot}5\times10^{10}$ vp (n=6)	5×10^{10} vp (n=6)	All groups (n=15)
Sex				
Female	1 (33%)	4 (67%)	4 (67%)	9 (60%)
Male	2 (67%)	2 (33%)	2 (33%)	6 (40%)
Age, years	25 (20–29)	27 (21–31)	24·5 (20–38)	25 (20–38)
Ethnicity				
White	2 (67%)	5 (83%)	5 (83%)	12 (80%)
Asian	0	0	1 (17%)	1 (7%)
Black	0	1 (17%)	0	1 (7%)
Mixed White and Black Caribbean	1 (33%)	0	0	1 (7%)

Data are n (%) or median (range). vp=virus particles.

Table 1: **Baseline characteristics**

June 11, 2021, and Jan 13, 2022 (figure 1; table 1). Nine (60%) of 15 volunteers were female and six (40%) were male (table 1). Three participants were allocated to the low-dose group (5×10^9 vp), six to the intermediate-dose group ($2\cdot5\times10^{10}$ vp), and six to the high-dose group (5×10^{10} vp; figure 1). All participants received a single dose of ChAdOx1 RVF vaccine according to their allocated group and were followed up until the final timepoint at day 84. The final follow-up visits occurred on April 6, 2022. No major protocol deviations occurred. Four timepoint-related protocol deviations occurred relating to attendance of visits outside of the planned schedule, including three participants attending the day 28 visit between 6 and 11 days later than scheduled and one participant attending the day 56 timepoint 17 days earlier than scheduled.

ChAdOx1 RVF had an acceptable safety and tolerability profile during interim safety reviews, allowing dose-escalation to proceed as planned. No serious adverse events occurred in any of the participants after vaccination. Mild local reactions were common, with 14 (93%) of 15 participants reporting solicited local adverse reactions (table 2). One participant vaccinated with the intermediate ChAdOx1 RVF dose reported moderate local symptoms (ie, redness, injection site pain, and warmth) occurring from day 2 to day 5 after vaccination, but all other participants reported only mild local symptoms. Injection-site pain was the most frequently occurring local adverse reaction, occurring in 13 (87%) of 15 participants (table 2). Local reactions primarily occurred in the early post-vaccination period, with a median onset time of 1 day (IQR 0–1) after vaccination and median duration of 2 days (1–5; appendix 2 p 3).

No participants in the low-dose group reported any systemic solicited adverse events. However, four (67%) of six participants in the intermediate-dose group and all participants in the high-dose group reported systemic symptoms that were mostly mild in intensity (table 2). Systemic reactions were transient and self-limiting, with a median onset time of 1 day (IQR 0–1) after vaccination and median duration of 1 day (1–3). The most common systemic reactions were headache and fatigue (table 2). Fever after vaccination (defined as a temperature ≥38°C), self-measured with a provided home thermometer, occurred transiently in two participants in the high-dose group (who also reported subjective feelings of feverishness) but not in other groups (table 2). An additional two participants in the intermediate-dose group reported either mild or moderate subjective feelings of feverishness, although with normal measured temperatures (table 2).

All unsolicited adverse events reported within 28 days of vaccination were either mild or moderate in severity (appendix 2 pp 4–5). Gastrointestinal symptoms were the most common unsolicited adverse reactions (assessed as at least possibly related to vaccination), occurring in four (27%) of 15 participants, and were mostly mild in severity, although one participant in the intermediate-dose group reported moderate severity diarrhoea and vomiting on day 1 after vaccination that resolved within 24 h. Additional moderate severity-related adverse reactions were lower-limb muscle cramps occurring on day 1 after vaccination only (occurring in one participant in the intermediate-dose group) and worsening of pre-existing dysmenorrhoea symptoms (occurring in one participant in the intermediate-dose group). Other

	5×10^9 vp (n=3)			$2\cdot5\times10^{10}$ vp (n=6)			5×10^{10} vp (n=6)		
	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Any solicited symptom	2 (67%)	0	0	4 (67%)	2 (33%)	0	5 (83%)	1 (17%)	0
Any local symptom	2 (67%)	0	0	5 (83%)	1 (17%)	0	6 (100%)	0	0
Pain	2 (67%)	0	0	4 (67%)	1 (17%)	0	6 (100%)	0	0
Redness	0	0	0	1 (17%)	1 (17%)	0	1 (17%)	0	0
Warmth	2 (67%)	0	0	4 (67%)	1 (17%)	0	1 (17%)	0	0
Itch	1 (33%)	0	0	2 (33%)	0	0	0	0	0
Any systemic symptom	0	0	0	2 (33%)	2 (33%)	0	5 (83%)	1 (17%)	0
Fever	0	0	0	0	0	0	1 (17%)	1 (17%)	0
Feverishness	0	0	0	1 (17%)	1 (17%)	0	1 (17%)	1 (17%)	0
Arthralgia	0	0	0	3 (50%)	0	0	2 (33%)	0	0
Myalgia	0	0	0	2 (33%)	0	0	2 (33%)	0	0
Fatigue	0	0	0	3 (50%)	1 (17%)	0	2 (33%)	1 (17%)	0
Headache	0	0	0	3 (50%)	1 (17%)	0	4 (67%)	1 (17%)	0
Nausea	0	0	0	2 (33%)	1 (17%)	0	0	0	0
Malaise	0	0	0	2 (33%)	2 (33%)	0	1 (17%)	1 (17%)	0

Data are number and percentage of participants reporting local and systemic solicited adverse events within 7 days of vaccination with ChAdOx1 RVF. The maximum severity of each adverse event reported by individual participants is shown. The maximum severity of any solicited symptom, any systemic solicited symptoms, and any local solicited symptoms reported by individual participants is also shown. RVF=Rift Valley fever. vp=virus particles.

Table 2: **Solicited adverse events within 7 days of vaccination with ChAdOx1 RVF**

related adverse reactions included local reactions, such as mild lymphadenopathy, vaccine site discomfort, vaccine site joint discomfort, and a report of moderate axillary pain, each reported in one participant in the intermediate-dose group.

Mild COVID-19 occurred in two participants within 28 days of vaccination, both in the high-dose group. The first of these participants tested positive on a COVID-19 lateral flow device after mild feverishness on day 1 after vaccination. Their symptoms resolved by day 2 and the participant was otherwise well. The second of these participants tested positive for COVID-19 after having mild to moderate upper respiratory tract symptoms (eg, sore throat, sneezing, and cough) at day 20 that resolved by day 27. Neither of their immune responses to Rift Valley fever virus Gn and Gc was remarkable compared with other participants and how, if at all, their COVID-19 infections affected their immune response is unclear.

Laboratory adverse events are described in appendix 2 (p 6). A day 2 haematology sample was missing for one participant in the high-dose group, so clinical haematology bloods were only analysed for five participants in the high-dose group at that timepoint. Transient decreases in total white cell count occurred at day 2 in three (50%) of six participants in the intermediate-dose group and one (20%) of five participants in the high-dose group. Transient decreases in lymphocyte count occurred at day 2 in two (33%) participants in the intermediate-dose group and one (20%) participant in the high-dose group. Transient decreases in neutrophil count occurred at day 2 in one (17%) participant in the intermediate-dose group and two (40%) participants in the high-dose group. Lymphopenia graded as severe occurred in two participants in the high-dose group, both occurring at a single timepoint only (day 2 or 7). These adverse events fully resolved at the subsequent follow-up visit timepoint in both cases. No platelet count abnormalities were seen in any participants during the study. Hypokalaemia graded as severe was recorded at a single timepoint (day 28) in one participant who had otherwise typical clinical biochemical markers and was well. This hypokalaemia was assessed as not causally related to vaccination and attributed to pseudohypokalaemia by investigators due to the use of lithium heparin blood tubes and a 12 h delay from venepuncture to clinical blood sample processing.

ChAdOx1 RVF was highly immunogenic, with 12 (80%) of 15 participants having a detectable Rift Valley fever neutralising antibody response that peaked at day 28 after vaccination and persisted to the final follow-up visit at day 84 (figure 2). The three participants who did not have a neutralising antibody response were either in the low-dose (n=2) or intermediate-dose group (n=1; figure 2). Binding IgG titres targeting Gc peaked at day 28, remaining high during the 3-month follow-up period (figure 2). This sustained level was predominantly affected by strong IgG1 and IgG3 responses, with no substantial induction of IgG2 and IgG4 detected (appendix 2 p 7). There was a strong correlation between titres of binding IgG targeting Gc and their ability to neutralise Rift Valley fever virus (Spearman correlation, r=0·84, 95% CI 0·55 to 0·95; appendix 2 p 8). Binding IgG titres towards Gn were comparatively low, with a modest increase in the median response after vaccination in all dose groups (figure 2). This modest increase was emphasised by minimal detection of any increase in the IgG subclasses after vaccination and poor correlation with neutralising antibody titres (r=0·39, –0·17 to 0·76; appendix 2 p 7). Two participants, from the low-dose (n=1) and high-dose groups (n=1), had consistently high titres of non-specific IgG binding Gn, with prevaccination responses being higher than the median peak response after vaccination; neither increased their response after vaccination. These participants showed no neutralising activity at day 0, a highly specific assay, suggesting that substantial cross-reactivity could explain the high Gn ELISA background.

ChAdOx1 RVF elicited a dose-dependent IFNγ ELISpot response in 14 (93%) of 15 participants (figure 2). Only one participant in the low-dose group did not have any detectable IFNγ response. Responses in the low-dose group peaked at day 28 after vaccination, with a median

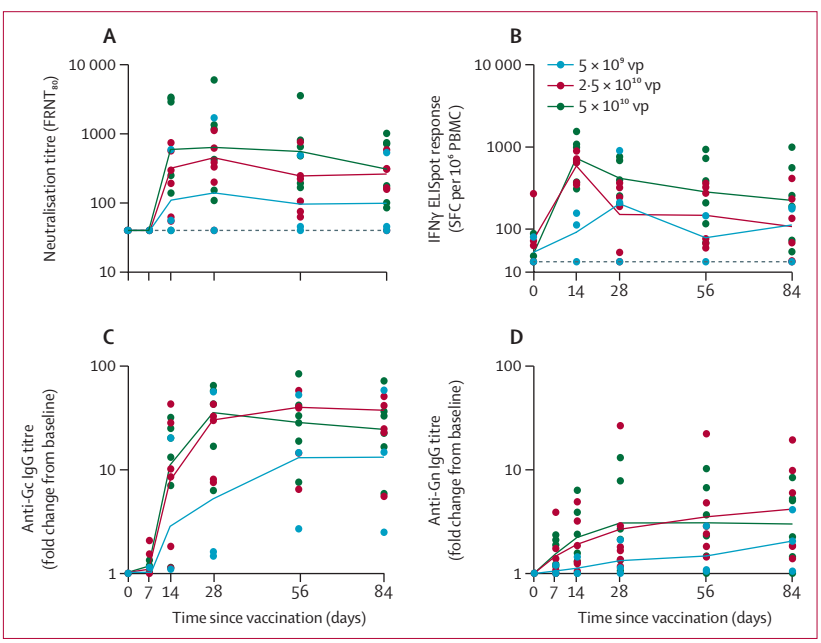

Figure 2: **Humoral and cellular responses generated by ChAdOx1 RVF vaccination**
ChAdOx1 RVF vaccine immunogenicity kinetics are shown for all participants (n=15) by dose allocation and at all immunology sampling timepoints during the 3 months of follow-up. (A) Rift Valley fever neutralising antibody titres. Dashed lines represent the lower limit of detection. (B) Summed Gn and Gc IFNγ ELISpot responses. Dashed lines represent the lower limit of detection. (C) Total IgG response against Gc shown as fold change from baseline. (D) Total IgG response against Gn shown as fold change from baseline. Circles represent mean values from three replicates. Lines represent median values. Four samples were obtained outside the defined timepoint periods from the $2{\cdot}5 \times 10^{10}$ vp group (day 28 sample taken 10 days after the timepoint [n=1], day 56 sample taken 17 days before the timepoint [n=1]) and the 5×10^{10} vp group (day 28 sample taken 6 days after the timepoint [n=1], day 28 sample taken 11 days after the timepoint [n=1]). ChAdOx1 RVF=chimpanzee adenovirus-vectored Rift Valley fever. $FRNT_{80}$=80% focus reduction neutralisation titre. PBMC=peripheral blood mononuclear cell. RVF=Rift Valley fever. SFC=spot forming cells. vp=virus particles.

of 212 spot-forming cells (SFC) per 10^6 peripheral blood mononuclear cells (PBMC; IQR 126–556). All participants in the intermediate-dose group had a response that peaked at day 14 after vaccination with median responses of 655 SFC per 10^6 PBMC (437–706); all participants in the high-dose group had a response that peaked at day 14 after vaccination with median responses of 810 SFC per 10^6 PBMC (441–1054; figure 2). IFNγ responses were broad, with all Gn and Gc peptide pools stimulating an IFNγ response in at least one participant (appendix 2 p 7). Overall, peak IFNγ responses were higher in magnitude towards Gn than they were towards Gc (appendix 2 p 7). There was no significant correlation between IFNγ ELISpot response and neutralising antibody titre (r=0·29, 95% CI –0·30 to 0·72; appendix 2 p 8). Of the three participants who did not develop neutralising antibody activity, one (from the low-dose group) showed no IFNγ response and two (one from the low-dose group and one from the intermediate-dose group) had comparatively strong IFNγ responses (figure 2).

Discussion

There are currently no vaccines for use against Rift Valley fever in humans, leaving the world susceptible to public health emergencies associated with Rift Valley fever epidemics. For this reason, WHO has prioritised development of Rift Valley fever countermeasures and compiled a target product profile to guide vaccine development.[6] In this study, we showed that ChAdOx1 RVF meets many of the optimal product characteristics listed in the WHO target product profile for a human Rift Valley fever vaccine,[6] including a favourable safety profile with predominantly mild or transient adverse effects, a rapid onset of Rift Valley fever neutralising antibodies, and cellular immunity within 2 weeks of single-dose vaccination. Adverse events observed in this first-in-human trial were consistent with the known safety profile of ChAdOx1 and other adenoviral vectored vaccines, and primarily consisted of mild local or systemic reactions generally starting and resolving within 48 h of administration of the vaccine. The highly transient but severe lymphopenia observed in two participants in the high-dose group has frequently been reported with other vaccines, appears to be a benign occurrence, and has been suggested to be caused by the redistribution of lymphocytes into lymphoid tissue.[19] These safety and immunological attributes of ChAdOx1 RVF after single-dose vaccination are crucial for reactive vaccination during Rift Valley fever outbreaks.[6] Its inherent thermostability, allowing storage at fridge temperatures for at least 1 year without loss of potency, should allow relatively easy deployment.[20]

A scalable manufacturing process for ChAdOx1-vectored vaccines has been developed and successfully used for the ChAdOx1 nCoV-19 (AZD1222) COVID-19 vaccine, which has now been used in more than 180 countries;[17] this process should be readily applicable for ChAdOx1 RVF. With a large proportion of the world's population having received ChAdOx1 nCoV-19, including in areas where Rift Valley fever vaccines are likely to be useful, there is a legitimate concern that multiple doses of homologous viral vectors could be problematic due to the build-up of antivector immunity. However, evidence from previous ChAdOx1 vaccine trials suggests that neither previous doses of ChAdOx1 vaccines or naturally acquired anti-ChAdOx1-vector neutralising antibodies lead to impaired immune responses to the encoded antigen.[21]

Post-marketing surveillance of adenovirus-vectored COVID-19 vaccines, including ChAdOx1 nCoV-19, uncovered a very rare association with thrombosis with thrombocytopenia syndrome. The biological mechanism of thrombosis with thrombocytopenia syndrome is incompletely understood, and whether it is also associated with adenovirus-vectored vaccines that do not deliver coronavirus antigens remains unknown.[22] Very few cases of thrombosis with thrombocytopenia syndrome have been reported outside of North America, Europe, and Australia, despite widespread use of adenovirus-vectored COVID-19 vaccines around the world. This regional disparity in incidence might, in part, represent under-reporting due to difficulties in identification and pharmacosurveillance; in some countries outside of North America, Europe, and Australia with excellent pharmacovigilance, thrombosis with thrombocytopenia syndrome appears to be very rare.[22]

In keeping with our previous livestock studies,[16,18] a single dose of ChAdOx1 RVF elicited high neutralising antibody titres in humans, with the highest titres being observed in the high-dose group (5×10^{10} vp), which is likely to be the preferred dose for later phase studies. The neutralising antibody response persisted to the end of follow-up at 3 months in all individuals who had seroconversion after vaccination, irrespective of vaccine dose. Although the precise neutralising antibody titre that correlates with protection against Rift Valley fever is yet to be established, it appears to be very low, with only few instances in the literature of an animal or human carrying Rift Valley fever neutralising antibodies of any level developing clinical Rift Valley fever.[16,23–25] Studies attempting to investigate the minimal protective neutralising antibody titre with rodent models have substantiated this evidence. An early study in hamsters after vaccination or adoptive transfer with human immune serum found that neutralising antibody titres (measured by plaque reduction neutralisation test [PRNT]) of between 1/10 and 1/20 offered full protection.[26] A more recent study of adoptive transfer of serum from human MP-12 vaccine recipients into mice[27] showed PRNT titres as low as 1/5 seem largely protective. Our own data from livestock vaccinated with ChAdOx1 RVF have shown complete protection from challenge, even in animals with titres that are orders of magnitude lower

than the highest responders.[16] Unfortunately, comparisons of neutralising antibody titres elicited after different Rift Valley fever vaccine candidates are difficult without harmonised assays or a shared serum standard. Our laboratory is, however, among several participating in the establishment of the first WHO International Standard for anti-Rift Valley fever virus antibody, which will facilitate comparisons with different vaccine candidates in the future. These data show the use of viral envelope glycoproteins Gn and Gc (both targets of neutralising antibodies)[8,28] as the main components of candidate Rift Valley fever vaccines in development.[5,6,10] These envelope glycoproteins are important for viral attachment and entry into cells and have little genetic diversity, such that neutralising antibodies generated by vaccination or natural infection provide cross-protection against heterologous virus strains or lineages.[8,29]

The short follow-up duration of this first-in-human trial precluded durability assessments of immune responses generated by vaccination. However, the high neutralising antibody titres and the robust IFNγ cellular response detected within 2 weeks after vaccination bode well for reactive use of the vaccine during Rift Valley fever outbreaks in which rapid induction of immunity is necessary to protect individuals at the highest risk of exposure.[6] Future phase 2 trials with long-term follow-up in populations living in settings prone to Rift Valley fever epidemics will establish the durability of the vaccine-induced neutralising antibody response, its relationship with T-cell responses, and whether homologous prime-boost regimens would provide any benefit in the magnitude of the memory B-cell frequencies compared with the single-dose regimen. The relevance of the observed predominance of anti-Gc humoral response to vaccination, despite a strong IFNγ response targeting both Gn and Gc peptides, will need further investigation in future studies. Whether a particular neutralising antibody titre is made up primarily of antibodies targeting Gc rather than Gn or targeting Gn rather than Gc is unlikely to be of consequence regarding efficacy. Although Gn is being expressed as evidenced by the IFNγ ELISpot response, its tertiary structure might differ from the native protein as it appears on the virion surface. However, if Gc expressed from the ChAdOx1 RVF vaccine is more accessible than its counterpart on the native virion surface, inducing antibodies predominantly towards Gc might be inevitable. Comparisons between immunity induced by vaccination and immunity acquired from natural Rift Valley fever infection will help to define key mechanisms of protection[8] and establish whether hybrid immunity has an effect on immunological attributes of the memory B-cell response, as observed for SARS-CoV-2.[30]

This first-in-human trial had several limitations, including the small sample size that was sufficient for informing decisions on further evaluation in phase 2 trials but not for detection of any rare adverse events. Subsequent trials will allow assessment of any rare adverse events associated with vaccination. Long-term durability of the immune response could not be established due to the short follow-up duration. Furthermore, the study participants were predominantly White and from a population in which Rift Valley fever is not endemic. The four visits conducted outside of the planned trial visit schedule might also have added variability to the measured immune responses. ChAdOx1 RVF and other candidate human Rift Valley fever vaccines also require evaluation in regions endemic for Rift Valley fever. In addition to this trial, ChAdOx1 RVF is currently being investigated in an ongoing phase 1b trial in healthy adults in Uganda (NCT04672824). However, to generalise vaccine performance to populations at most risk of Rift Valley fever, further trials of ChAdOx1 RVF should also focus on evaluating its use in individuals involved in animal husbandry in endemic regions, which might include children and adolescents, and also in people who are pregnant in whom risk of miscarriage associated with Rift Valley fever is high.[4]

Contributors

GMW, DJ, and DW conceptualised the study. PMF, AVSH, GMW, DJ, DW, and AL created the methodology. DJ, PMF, AP, IP, DW, JNG, HKK, and DM did the investigation. KJE acquired resources. JNG, HKK, DM, and DW validated the data. DJ, DW, and CT curated the data. The study was supervised by TAB, SCG, and GMW. AL, AB, and NT did project administration. GMW, AVSH, BC, and PK acquired funding. DJ, DW, and GMW wrote the original draft. DW and DJ accessed and verified all data in this study and did the formal data analysis. All authors reviewed and edited the report and approved the final version.

Declaration of interests

PMF receives funding from the Brazilian Government (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for PhD work and consulting fees from Vaccitech, a company developing ChAdOx1 vectored vaccines. KJE is named as a contributor to a patent relating to ChAdOx1 MERS. TAB receives funding from the Medical Research Council UK. SCG is named as an inventor on the patent covering ChAdOx1 use as a vaccine vector and holds stock in Vaccitech. AVSH has received royalties from the COVID-19 vectored ChAdOx1 vaccine to both himself and his institution, and is named as an inventor on the patent covering ChAdOx1 use as a vaccine vector. All other authors declare no competing interests.

Data sharing

Deidentified participant data will be made available upon request to the Chief Investigator (AVSH) with publication. Proposals will be reviewed and approved by the sponsor, Chief Investigator, and collaborators on the basis of scientific merit. After the approval of a proposal, data can be shared through a secure online platform after signing a data access agreement. The trial protocol and statistical analysis plan are included in appendix 2.

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