Development and evaluation of a live attenuated camelpox vaccine from a local field isolate of the virus

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Summary

A strain of camelpox virus (CMLV) isolated in the Sudan was attenuated by serial passage in Vero cell monolayers for use as a future vaccine strain. The safety and potency of passage 115 virus (designated Sudan CMLV/115) was tested. Camels inoculated with CMLV/115 showed no clinical disease or skin lesions, developed low-level antibodies and cell-mediated immune response and resisted challenge with virulent wild-type CMLV. Field testing of the candidate vaccine showed that the developed vaccine induces immune response and is safe for young and pregnant camels.

Keywords

Camelpox – Camelpox virus – Cell culture attenuation – Vaccine – Vaccine evaluation.

Introduction

Camelpox is a contagious skin disease of camelids caused by camelpox virus (CMLV). It is characterised by mild local skin infection and, less frequently, severe systemic infections. The disease is considered the most important infectious disease in Old World camelids, and, from an economic point of view, it is possibly the most important remaining orthopoxvirus (OPV) disease (1, 2, 3). CMLV belongs to the family Poxviridae (4, 5).

Camelpox was initially described in Punjab, India, in 1909 (6). Subsequently, outbreaks have been reported in many countries of the Middle East, Asia and Africa, and in southern Russia, where the disease is enzootic. In the Sudan, the disease occurred in epizootics that lasted for 2 to 5 months, with higher prevalence in winter. The mean morbidity and mortality rates in camel calves of less than one year old were 60.2% and 8.8%, respectively (7).

Unfortunately, effective control programmes such as sanitary measures, quarantine of infected areas, restriction of camel movements, management of clean drinking water and avoidance of skin abrasions are difficult to apply owing to the migratory pattern of camels and the difficulty of reaching the animals, especially during the rainy season (8). As a result of major economic losses from numerous camelpox outbreaks, research has been oriented towards the development of prophylactic methods to contain the spread of camelpox in enzootic countries.

Few camelpox candidate vaccines have been developed. They contain the following CMLV strains: Jouf-78 (9), VD47/25 (10), Ducapox 298/89 (11) and CMLV-T8 (12). Ducapox live attenuated vaccine is manufactured in South Africa, T8 killed vaccine in Morocco, and the Jouf-78 has been used to produce live attenuated vaccine in Saudi Arabia (9). Recently, it was discovered that the Jouf-78 vaccine contained a vaccinia virus (VACV) rather than CMLV (13); consequently, the production of this vaccine has ceased.

The objective of the present research work was to make available a new attenuated strain of CMLV for disease eradication, as very few attenuated CMLV strains are available, most have not been sufficiently tested and the identity of one has been mistaken. Furthermore, vaccination against camelpox is not popular and is not yet practised in East Africa. It is anticipated that local production of camelpox vaccine in a low-income country such as Sudan, which has more than four million camels, will improve camel production and support its economy through the export of vaccinated camels.

Materials and methods

Camelpox virus strains

The CMLV used to produce a live attenuated vaccine was a pathogenic field strain (CP/Dbg/92/3) isolated from sick camels during a field outbreak that occurred in the Butana area of Eastern Sudan (14). CMLV strain VD 45, kindly provided by the French Agricultural Research Centre for International Development (CIRAD), was used to prepare antigen for the delayed hypersensitivity test (DHT). For the challenge experiment, the pathogenic field strain (CP/NW/92/2) (14, 15) in its 3rd cell culture passage was used.

Dromedary camels

Camels 12 to 18 months old, with no history of camelpox were obtained from the Camel Research Centre in the Faculty of Veterinary Medicine at the University of Khartoum. They were kept at the animal house in the Department of Microbiology where the experiment was performed. Camels were treated against ticks and internal parasites using Ivermectin.

Virus attenuation

The CP/Dbg/92/3 strain of CMLV was serially propagated in confluent monolayers of Vero cells grown in $25\,cm^2$ tissue culture flasks. The virus was passaged for a total of 115 passages according to a standard technique (3). Passages 50 (CMLV/50), 100 (CMLV/100) and 115 (CMLV/115) viruses were used in the evaluation

experiments. During attenuation the virus was clone-purified three times at passages 30 and 90 by the limiting dilution technique, according to the method described by Downie and Haddock (16), the procedure was repeated twice. The supernatant of each passage was examined to determine whether or not it was free from bacterial and fungal contamination by inoculation in thioglycolate broth, mycoplasma agar base enriched with supplements, and Sabouraud's agar, according to standard techniques (17).

Preparation of experimental vaccine batch

Master seed, working seed and the experimental vaccine batch were prepared using Vero cells according to the World Organisation for Animal Health (OIE) principles for the production of veterinary vaccines (18).

Virus identification

The CMLV in the virus preparations used in this study was identified using the *alpha* virus neutralisation procedure (constant-serum, diluted-virus) as described by Beard (19). Each virus was simultaneously tested against negative and anti-CMLV hyper-immune serum previously produced in rabbits against the VD_{45} strain of CMLV (10). Additionally, each virus preparation was tested by polymerase chain reaction (PCR) following the procedure described by Sheikh *et al.* (15).

Determination of immune response

Sera collected from experimental dromedary camels were heat-inactivated at 56°C for 30 min and tested for circulating antibodies against CMLV using the *Beta* serum neutralisation test (SNT) (constant-virus, diluted-serum) following a standardised procedure (3).

Titration of virus infectivity

The infectivity titre of each virus preparation was analysed using Vero cells (20) and the tissue culture infective dose 50/ml ($TCID_{50}$/ml) determined (21).

Efficacy test in dromedary camels

Eight experimental camels were divided into four groups (A, B, C and D) of two animals each. Each animal in group A received a subcutaneous injection with 1 ml of CMLV/115 suspension ($10^{3.8}TCID_{50}$/ml), group B received a subcutaneous dose of $1 \times 10^{5.8}$ TCID and group C $3 \times 10^{5.8}$; group D was inoculated with phosphate-buffered saline as a control (Table I). Experimental camels were followed daily for clinical signs and rectal temperature was recorded early morning for four weeks post vaccination.

Kinetics of antibody response in camels

Blood samples were drawn into plain tubes from the jugular vein of each animal on a weekly basis at the time of clinical examination. Sera were separated and stored at 4°C until tested for circulating antibodies by SNT, as described above.

Total leukocyte count and differential leukocyte count

Blood samples were collected from the experimental animals on a weekly basis in EDTA to estimate the total leukocyte count (TLC) and the differential leukocyte count (DLC). Samples were analysed within 30 min of collection (post vaccination and post challenge) using standard haematological techniques (22).

Delayed hypersensitivity test

Cell-mediated immune response was measured using a DHT according to the method described by Khalafalla and El Dirdiri (23).

Challenge experiment

The challenge experiment was carried out on day 42 post vaccination by subcutaneous inoculation of $10^{5.6}$ TCID$_{50}$/ml of the CP/NW/92/2 strain of CMLV in a shaved area on the left side of the neck. All eight experimental camels, including the control non-vaccinated animals, were challenged. The animals were closely monitored for the development of specific disease or appearance of any reaction at the site of inoculation. Rectal temperature was recorded daily up to day 14 post challenge and serum samples were collected from the camels at days 0, 7 and 14 post challenge.

Field vaccination trial using CMLV/115

The field vaccination trial, which included 44 animals of different ages and sex, was carried out in a camel herd in the outskirts of Khartoum city in Sudan. Camels were vaccinated with CMLV/115 ($10^{5.8}$TCID$_{50}$/ml) subcutaneously. The camels in the herd were divided into two groups on the basis of age: young animals (<4 years; $n = 28$) and adult animals (>4 years; $n = 16$). Pre-vaccination sera were collected and tested for antibodies against CMLV by SNT. Two pregnant camels were included in the trial. Camels were followed every other day for clinical signs for five weeks post vaccination. Blood samples were collected weekly and sera were tested for circulating antibodies by SNT as described above.

Results

Development of live attenuated camelpox virus vaccine

A stock virus was prepared from the wild-type CMLV. The virus was identified by virus neutralisation test and PCR. The virus was then serially propagated in confluent monolayers of Vero cells. At passage 115 the TCID$_{50}$/ml was found to be $10^{5.5}$/ml and the cytopathic effect appeared as cell rounding, plaque formation and complete destruction of the monolayer within 48 h post inoculation (PI).

Bacterial, fungal and mycoplasma sterility tests

Freedom from bacteria, fungi and mycoplasma contamination in virus stock, low and high passage virus preparations and the experimental vaccine batches was certified by the absence of any growth on selective media.

Efficacy test on dromedary camels

Post-vaccination reaction

Neither the camels vaccinated with different doses of CMLV/115 nor the contact controls showed any local lesion at the site of inoculation. Clinically, throughout the experiment, experimental animals appeared healthy and behaved normally, without any clinical signs.

Kinetics of immune response

Freedom from circulating antibodies in the experimental camels prior to inoculation with CMLV/115 was confirmed by SNT. Formation of neutralising antibodies against CMLV was detected in the vaccinated camels in the first week PI. While camels inoculated with a low dose showed relatively lower antibody titre, no significant variation was observed in the humoral response of camels inoculated with $1 \times 10^{5.8}$ and $3 \times 10^{5.8}$ $TCID_{50}$/ml of the CMLV/115. On the other hand, no neutralising antibodies were detected in the sera of the control camels (Fig. 1). Camels inoculated with a dose of $1 \times 10^{3.8}$ developed a comparatively lower immune response. However, the immune response of camels inoculated with $1 \times 10^{5.8}$ was comparable to that of the camels that received three times that dose ($3 \times 10^{5.8}$).

Delayed hypersensitivity test

Camels vaccinated with CMLV/115 reacted positively to DHT, whereas the non-vaccinated control camels showed no increase in skin thickness at the site of inoculation. Twenty-four hours later, a two to threefold increase in the skin thickness was detected in vaccinated animals, which regressed on the fourth day PI (Fig. 2).

Total leukocyte count

Vaccinated camels showed a normal TLC following immunisation and subsequent challenge with the virulent virus. However, a moderate leukopoenia was noticed in non-vaccinated camels seven days post challenge.

Differential leukocyte count

Vaccinated camels showed a normal DLC following immunisation and subsequent challenge with the virulent virus. However, a slight lymphocytopoenia was noticed in non-vaccinated camels seven days post challenge.

Challenge experiment

Experimental camels were challenged with the pathogenic CMLV field strain CP/NW/92/2. Vaccinated animals remained healthy, there was no detectable rise in body temperature and the feeding behaviour was normal for up to 30 days post challenge. Control dromedary camels displayed local pox lesions at the site of inoculation three days post challenge, accompanied with enlarged regional lymph nodes. A rise in rectal temperature up to 41.3ºC was recorded on the fifth day post challenge. Lesions were observed on other parts of the body, particularly on the thigh, neck and forelimbs. There was formation of papules on the fifth day post challenge, which turned into vesicles and pustules on the seventh day, oozing of fluid on day nine and scab formation on day 14 post challenge.

Field vaccine efficacy trial

No post-vaccination clinical signs were observed among dromedary camels vaccinated with CMLV/115 during the experiment, which appeared healthy and behaved normally. Vaccination of pregnant she camels did not result in abortion or any other clinical signs. Antibody responses of camels indicated that some of the pre-vaccination serum samples of adult animals contained variable levels of neutralising antibodies against camelpox ranging from 1 to 2 ($\log_2$). All vaccinated animals showed specific antibody response at the first week post

vaccination, ranging from 2 to 3 ($\log_2$). Antibody response reached a plateau three weeks post vaccination, ranging from 3 to 5 ($\log_2$). Notably, adult animals with pre-vaccination antibodies induced relatively low titres (Fig. 3).

Discussion

Camelpox, a smallpox-like illness that occurs only in camels, could be eliminated through an intensive vaccination programme (4). It meets the basic requirements to be a candidate for eradication (24) and therefore represents a target for global eradication, which should be a priority because of the increased potential for transmission to humans since the suspension of smallpox vaccination (25).

In the present study, a local field CMLV was attenuated by serial passage in Vero cell monolayers for use as a future vaccine strain. The authors first tested the efficacy and safety of CMLV/100 by subcutaneous inoculation of eight susceptible dromedary camels (data not shown). Inoculated camels developed a local reaction: a small nodule confined to the site of inoculation that appeared on the third day post vaccination, dried up within seven days, and healed within three weeks of vaccination. Therefore, the authors passaged the virus 15 times more (CMLV/115). Camels inoculated with CMLV/115 remained apparently healthy without any adverse reactions, neither signs of illness nor a rise in rectal temperature were recorded for up to 40 days PI. Vaccinated camels developed a measurable immune response which reached a plateau at around day 21 PI.

Despite inducing a relatively weak primary antibody response, all vaccinated animals were protected when challenged with wild-type virus. Thus, either the low levels of neutralising antibodies were sufficient for protection, or the small number of antibody-producing cells generated during primary vaccination was sufficient to expand and to protect the animals upon challenge; alternatively, other components of the immune system, likely cell-mediated immunity, may have been compensating for this low antibody response (25). However, the induction of protection does not always correlate with the levels of neutralising antibodies induced upon vaccination by live

vaccines (26, 27). Camels inoculated with a low dose of the candidate vaccine showed lower kinetics of antibody production compared to those inoculated with a dose of $10^{5.8}$ and a high-titre virus. The high-titre virus induced no adverse clinical reaction, indicating that it is safe and that there is an absence of residual virulence.

In the DHT all vaccinated camels reacted positively, with a remarkable increase in skin thickness as compared with controls. This emphasised the role played by the cell-mediated immunity, the increase in thickness was relatively higher in camels vaccinated with the high-titre virus. This finding agreed with that of Khalafalla and El Dirdiri (23), who observed that live attenuated camelpox vaccines induce relatively higher cell-mediated immunity than killed vaccines. A normal TLC and DLC following immunisation and subsequent challenge with the virulent virus was detected. However, a moderate leukopoenia and a slight lymphocytopoenia were noticed in control camels seven days after being challenged with wild-type virus, similar results were obtained by Hussein and Al-Mufarrej (28).

Based on efficacy tests of three different doses of the candidate vaccine, a dose of $10^{5.8}$ $TCID_{50}$/ml was selected and used in a field vaccination trial. Results showed a seroconversion rate at the first week that ranged from 2 to 3 ($\log_2$) and reached a plateau three weeks post vaccination with a range between 3 and 5 ($\log_2$). Notably, adult animals with pre-vaccination antibodies induce relatively low antibody titres, which may be due to neutralisation of the vaccine virus by circulating antibodies. Khalafalla *et al.* (29), in a field serological survey, found that the prevalence of antibodies against camelpox was high (87%) in camels more than four years old, compared to young animals (40%). Results also showed that vaccination was safe for pregnant camels; these results were similar to those obtained by Khalafalla and El Dirdiri (23), who tested the efficacy and safety of a live attenuated (Ducapox, UAE) and a killed (T8, Morocco) camelpox vaccine.

From previous field studies, a single dose of vaccine (10^5 $TCID_{50}$) was recommended for full protection. The attenuated CMLV strain

VD47/25, passaged 80 times in cell culture, has also been evaluated as a potential camelpox vaccine (10). As observed during experiments in Mauritania, this strain was innocuous in camels (dose of $10^{4.7}$ $TCID_{50}$, subcutaneous administration) and protected them from severe and lethal CMLV infection. In the United Arab Emirates, a modified live camelpox vaccine obtained by passaging the strain CaPV298-2 in Vero cells has been used (30). This vaccine, called Ducapox, was used for field vaccination just before the onset of a large camelpox outbreak in Dubai in 1993–1994. Among 2,000 vaccinated camels, seven developed the disease, but it was not known if these animals were infected before the vaccination or if they were true vaccination failures (30). Further, Wernery and Zachariah (11) showed that a single vaccination protected camels for six years, although this was only shown with two animals. Vaccine efficacy has also been demonstrated in New World camelids against an otherwise lethal CMLV challenge (31). The safety and potency of Ducapox has also been explored in the study by Khalafalla and El Dirdiri (23), the vaccine requires subcutaneous injection, but a single dose ($10^{6.7}$) is enough to sustain protection for at least one year.

In the present study the absence of any indication of seroconversion in susceptible camels that were in contact with vaccinated camels might indicate that the viruses had not been excreted by vaccinated animals and/or transmitted to contact susceptible animals. Similarly, field testing revealed no evidence of seroconversion among non-vaccinated control animals, indicating the safety of the candidate vaccine in experimental camels. More field testing of the candidate vaccine and more detailed testing of residual virulence are needed.

Conclusion

A live attenuated candidate vaccine was developed from a local strain of CMLV in the Sudan and tested in terms of safety and potency in experimental camels and a small-scale field trial. The results of several tests indicated that, after being passaged 115 times, the candidate vaccine is potent, safe and has potential for controlling the disease.

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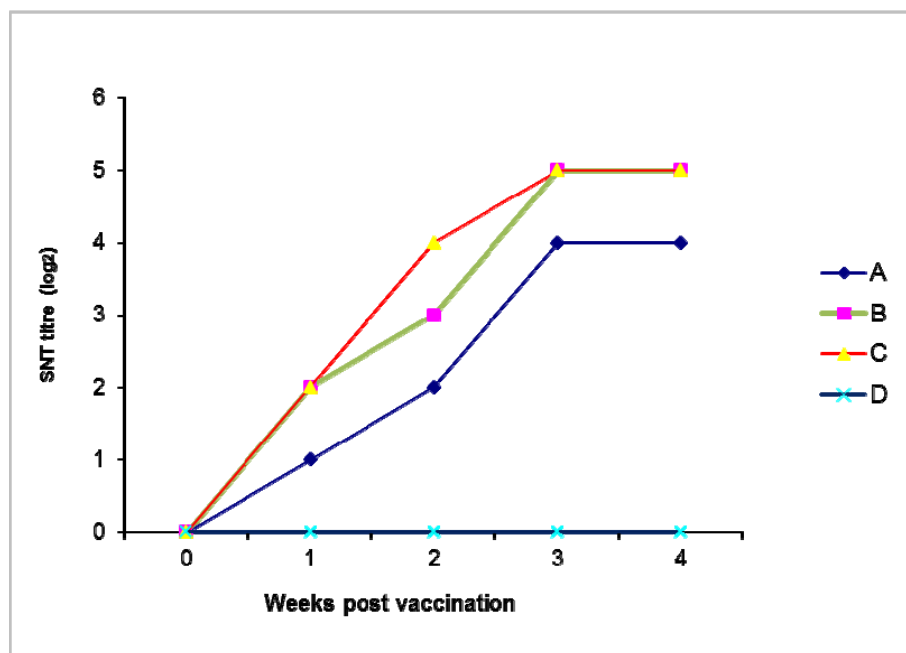
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Table I

Dose scheme followed to inoculate camels with CMLV/115

Group	Treatment ($TCID_{50}$)
A	$1 \times 10^{3.8}$
B	$1 \times 10^{5.8}$
C	$3 \times 10^{5.8}$
D	PBS (1ml)

PBS: phosphate-buffered saline
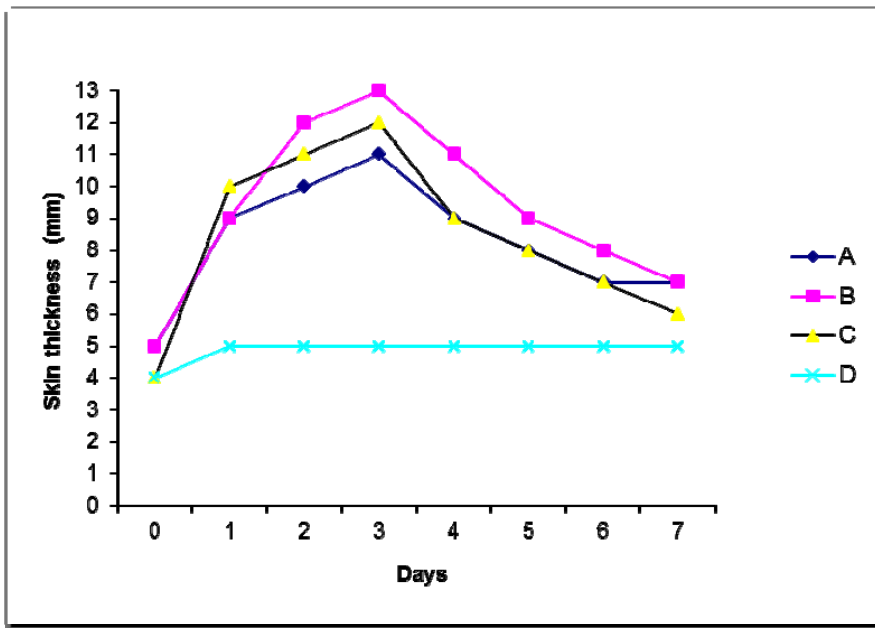
A: Camels inoculated with $10^{3.8}$TCID$_{50}$/ml

B: Camels inoculated with $10^{5.8}$TCID$_{50}$/ml

C: Camels inoculated with $3 \times 10^{5.8}$TCID$_{50}$/ml

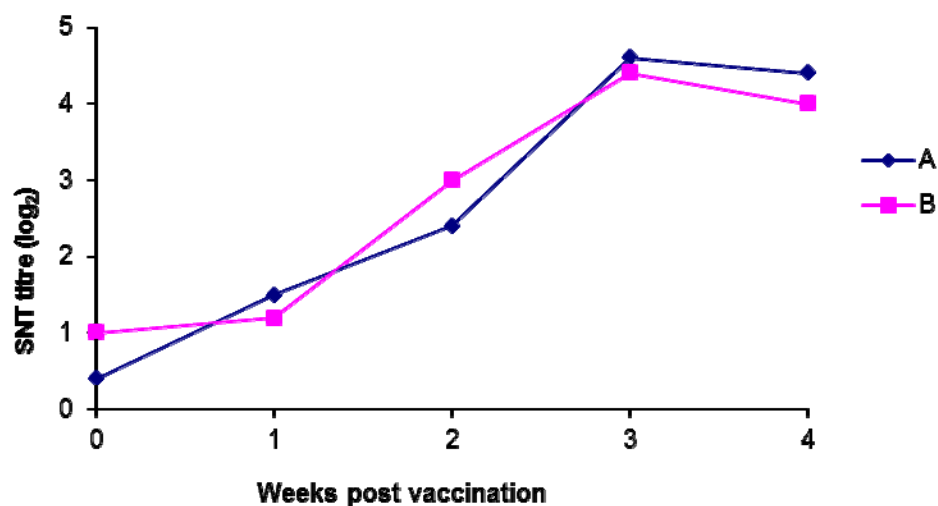D: Control camels inoculated with phosphate-buffered saline

Fig. 1

Kinetics of antibody response as measured by serum neutralisation test (SNT) in camels vaccinated with CMLV/115 by subcutaneous injection

A: Camels inoculated with 103.8$TCID_{50}$/ml

B: Camels inoculated with 105.8$TCID_{50}$/ml

C: Camels inoculated with 3 × 105.8$TCID_{50}$/ml

D: Control camels inoculated with phosphate-buffered saline

Fig. 2

Skin thickness (delayed hypersensitivity test reaction) in camels inoculated with CMLV/115

A: Young camels

B: Adult camels

Fig. 3
Field vaccination trial

Kinetics of antibody response as measured by serum neutralisation test in camels vaccinated with a dose of $10^{5.8}$ $TCID_{50}$ of CMLV/115 by subcutaneous injection