Improving Thermostability of A Candidate Live Attenuated *Camelpox* Vaccine

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Abstract: The main focus of this work was to determine the effect of serial passage of *camelpox* virus on thermostability, as well as to select a thermostable derivatives of the vaccine strain. First, the virulent, passage 50 and passage 100 strains were stored at 37, 4 and – 20°C for up to 90 days. The stability of the viral strains was tested, marked reduction of viral infectivity was noticed. Similar results were obtained during incubation at 4 and –20°C, while all strains completely lost infectivity at 37˚C within 30 days. It seemed that thermostability was affected during viral attenuation. Secondly, the vaccine virus was subjected to extreme heat selection at 56˚C for increasing time (10-50 min) for nine passages aiming at improving the heat stability. Thermostability testing at 56˚C for 30 and 60 min showed that treated vaccine lost 2 to 4 $\log_{10}$, while original vaccine virus lost 3 to 5 $\log_{10}$ of the infectivity titer. Improving thermostability would give the vaccine adequate short-term stability at non-freezing temperatures to support manufacturing, short-term shipping and storage and significantly enhance its utility in the control of a *camelpox* outbreak. *Corresponding Authormuazm20@hotmail.com; muazm20@yahoo.com

Keywords: *Camelpox* , Thermostability , Extreme heat selection

Introduction

Camelpox (CP) is considered as the most important infectious disease in old world camelids and from an economic point of view, is possibly the most important remaining orthopoxvirus disease (Jezek *et al*., 1983). The disease is caused by *Orthopoxvirus cameli* a member of the genus Orthopoxvirus (OPV) (Moss., 2001). It occurs in almost every country in which camel husbandry is practiced (Wernery and Zachariah., 1999). The disease is widely spread in all parts of the Sudan where camels are raised (Khalaf-alla *et al*., 1998). Reports of vaccines produced against CP have come from Saudi Arabia, UAE and Morocco (Wernery and Kaaden., 2002). Recently a candidate vaccine was developed in the Sudan (Abd-Ellatif., 2011). Thermostability is a property enabling a vaccine to withstand exposure to high temperatures without total loss of it is infectivity and with retention of immu-nogenicity. The effect of temperature on virus infectivity varied between *camelpox* virus (CPV) isolates (Borisovich and Orekov., 1966; Al Falluji *et al*., 1979; Nguyen *et al*., 1989). The virus is readily inactivated by heat, direct sun light, weak acids and alkalis, iodine and potassium permanganate (Mc-Grane and Higgins., 1985). Thermo-labile vaccines is a serious drawback for their efficient use in hot climatic enviro-nments. Besides the stability of the vaccine during storage, the process of production can also affect the final potency, as the stability of enveloped viruses can be compromised in cell culture bulks due to temperature (Coroadinha *et al*., 2006; Silva *et al*., 2011). The main objectives of the present study were to determine the effect of serial passage on thermostability during storage at both freezed, refrigerated and high relevant storage temperatures, as well as to select of a thermostable vaccine seed.

Materials and Methods

Camelpox vaccine

Live attenuated c*amelpox* vaccine was locally developed (Abdellatif., 2011) from a pathogenic field strain isolated from sick camels during a field outbreak in *Butana* area, eastern Sudan (Khalafalla *et al*., 1998).

Cell culture

Cell seed of African green monkey kidney cells (*Vero*) was kindly provided by the Central Veterinary Research Laboratories (CVRL), Soba. Sudan and used for virus propagation and titration. The cells were maintained at 37˚C in a humidified atmosphere of 5% CO_2 in air with Glasgow Modified Eagles Medium (GMEM) supplemented with 2mM glutamine. All media and supplements were from *Sigma* as follows: Growth and Maintenance media: GMEM x 5 concentration (200ml), 0.5% lactalbumin hydrolysate(25ml**),** 1%yeast extract (25ml**),** tryptose phosphate broth (25ml)**,** 7.5% $NaHCO_3$ (8ml)**,** Penicillin (10000units/ml)/ streptomycin 10mg/ml (1ml) and Fungizone 1ug/ml (1ml)**.** Double distilled water (DDW) was added to complete to one liter. For preparation of growth media 100ml of Fetal bovine serum (FBS) was added, 20ml for maintenance**.**

Camelpox virus. *(CPV)* strains

Three vials each of CPV strains namely virulent virus (CPV/WT), passage 50 (CPV/50) and passage 100 (CPV/100) were randomly selected, reconstituted with sterile phosphate buffered saline (PBS).

Sterility test

Viral suspensions were subjected to bacterial, mycoplasma and fungal sterility in Thioglycolate broth, Mycoplasma agar base enriched with supplements and Sabouraud's agar respectively.

Virus propagation

Viral strains were propagated by inoculation of 0.1ml of the virus suspention into 25-cm^2 tissue culture flasks. The flasks were incubated at 37˚C and examined daily with an inverted microscope. When the cytopathic effect (CPE) was 70 – 80%, the infected cultures were frozen at – 20˚C, thawed at 37˚C and the culture medium was clarified by low speed centrifugation (1500g/min for 15min), aliquoted and preserved at – 20˚C until it was used.

Thermostability testing of CPV strains

Viral strains were aliqouted into sterile *Eppendorf* tubes and incubated at 37, 4 and – 20°C for up to 90 days to test the effect of viral passages on thermostability.

Titration of virus infectivity

The titer of CPV strains were calculated on day 0, 10, 30, 60 and 90 according to the method of Reed and Muench. (1938) as described by Villegas and Purchase. (1983).

Improving thermostability

Thermostability testing

A portion (0.5ml) of the vaccine suspension (CPV/100) was heated at 56˚C for 30 and 60 min and subsequently titrated for virus infectivity.

Extreme heating

A portion (0.5ml) of the vaccine suspension was subjected to heating at 56˚C at increasing time for 10, 15, 20, 25, 30, 35, 40, 45 and 50 min; at intervals recovered viruses were propagated in 25-cm^2 tissue culture flasks. The flasks were incubated at 37˚C and examined daily with an inverted microscope. When the CPE was 70 – 80 %, the infected cultures were harvested and clarified by low speed centrifugation (1500g/min for 15min). The supernatant was used as an inocula for the subsequent passage.

Thermostability testing

A portion (0.5ml) of the treated vaccine suspension (passage 9) was heated at 56˚C for 30 and 60 min then it was titrated for virus infectivity.

Results:

Bacterial and fungal sterility tests:

Freedom from bacteria, mycoplasma and fungi was certified by the absence of any growth on selective media.

Thermostability testing:

Thermostability testing of viral strains at 37, 4 and –20˚C revealed marked reduction of viral infectivity. Similar results were obtained during incubation at 4 and –20°C, while all preparations completely lost infectivity at 37˚C after 30 days post storage, the wild type virus seemed to be relatively stable in comparison with passaged viruses. (Figure 1).

Improving thermostability:

Titration of the vaccine:

Untreated vaccine:

Untreated vaccine strain was titrated in *Vero* cells. The tissue culture infective $dose_{50}$/ml ($TCID_{50}$/ml) was found to be $10^{5.5}$ $TCID_{50}$/ml (Figure. 2).

Treated vaccine:

Treated vaccine strain was heated at 56˚C for 30 and 60min. The tissue culture infective $dose_{50}$% was found to be $10^{2.6}$ and $10^{0.8}TCID_{50}$/ml respectively (Figure 2).

Extreme heating:

The vaccine preparation was subjected to extreme heating at 56˚C for increasing time (10-50min), passage nine was then tested for thermostability.

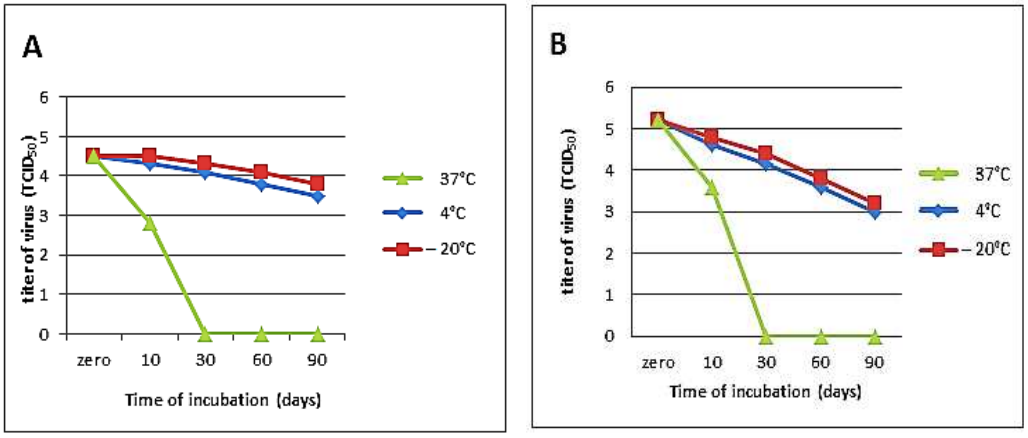
Thermostability testing:

Untreated passaged vaccine:

The final passage of the vaccine was titrated. The infectivity titer was found to be $10^{6.5}$ $TCID_{50}$/ml (Figure 2).

Treated passaged vaccine:

The final passage of the vaccine was heated at 56˚C for 30 and 60minutes. The infectivity titer was found to be $10^{4.5}$ and $10^{2.8}$ $TCID_{50}$/ml respectively (Figure 2).



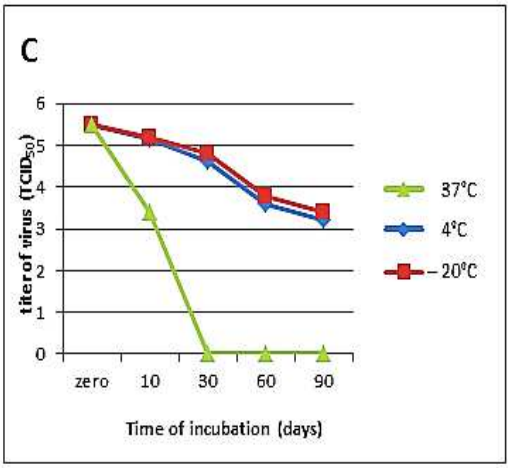


Fig 1**:** Thermostability testing of the viral preparations. **A.** CPV/WT **B.** CPV/50 **C.** CPV/100. Notice that all viral preprations completely lost infectivity at 37˚C after 30 days post storage.

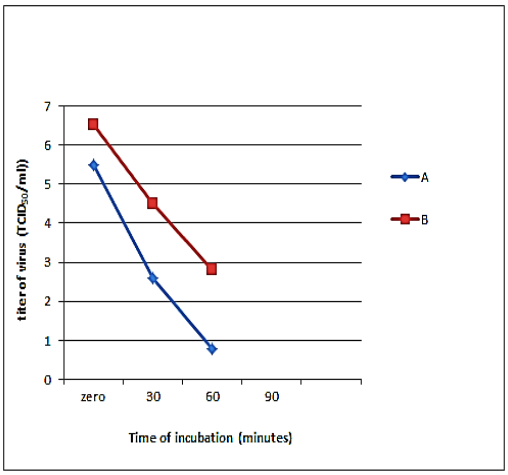


Fig 2: Thermostability testing of the original (A) and the treated vaccine virus (B) at 56˚C for 30 and 60mins.

Discussion:

Successful vaccination depends on several factors inherent to the vaccine, the immunogenic virus quality employed in its manufacturing, antigen stability, the amount of virus used per dose and the virus profile (Simi *et al*., 1970; Young *et al*., 2002). Conventional live vaccines have the disadvantage of the need to be kept at low temperatures to maintain their efficacy to provoke appropriate immune response, however, the cold chain maintenance during distribution can be very difficult in tropical countries, particularly in migratory pattern of camel production in the Sudan. This study was conducted to determine the effect of serial passage of *camelpox* virus on thermostability, as well to select a thermostable derivatives of the vaccine strain to retain the required potency during manufacuring and distribution insuring that

at least the minimum immunogenic titer is delivered. Thermostability testing of the viral passages indicated that viral stability was affected during attenuation. At 37˚C all preparations completely lost the infectivity 30 days post storage. Secondly, the vaccine strain was subjected to extreme heat selection at 56˚C for increasing time (10-50 min) for nine passages aiming at improving the vaccine stability. Thermostability testing at 56˚C for 30 and 60 min showed that treated vaccine lost 2 to 4log10 , while original vaccine virus lost 3 to 5log10 of the infectivity titer. No literature concerning *camelpox* vaccine stability testing and/or improving were so far available. The results were similar to those obtained by Nguyen *et al*. (1989) who found that CPV isolated in Niger is thermolabile at 56˚C for 10 min. However, conflicting data concerning stablility of virulent *camelpox* virus was described by Al Falluji *et al*. (1979). Varadarajan *et al*. (2000) support the use of short exposures to 56˚C to enhance the proportion of heat-resistant virus particles obtaining the seed for the next generation. According to the results of the present study it can be concluded that a degree (1log10) of improvement concerning tharmostability was attained in comparison with the original vaccine virus which could have a major impact in the efficacy and manufacturing costs of the candidate vaccine since higher virus titers with relatively higher stability can be achieved. The process needs to be validated to evaluate the effect of thermal selection on theromstability and other viral characteristics, particularly those essential to the maintenance of the viral immunogenicity.

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تحسين الثبات الحراري للقاح جدري الإبل الحي المضعف

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المستخلص:

هدفت الدراسة الي تحديد أثر تمرير فيروس جدري الجمال علي الثبات الحراري و إنتخاب عترات فيروسية تتميز بالثبات الحراري. أولا تم تخزين العترات الفروسية ، الفيروس الضاري، الفيروس التمرير 50 و الفيروس التمرير 100 في درجات حرارة 37، 4 و – 20°م. تم إختبار ثبات العترات الفيروسية، لوحظ إنخفاض واضح في مقدرة الفيروس علي إحداث العدوي. التخزين في 4 و – 20°م أفضي الي نتائج متشابهة، بينما في درجة 37°م كل العترات الفيروسية فقدت المقدرة الفيروس علي إحداث العدوي خلال 30 يوما. أظهرت الدراسة أن الثبات الحراري تأثر أثناء التضعيف الفيروسي. ثانيا تم تعريض الفيروس لدرجة حرارة مطلقة 56°م لفترات زمنية متزايدة (10–50 دقيقة) لتسعة تمريرات بغرض تحسين الثبات الحراري. عند إختبار الثبات الحراري في 56°م لمدة 30 و 60 دقيقة، إتضح أن معيارالفيروس الذي تم معاملته حراريا إنخفض 2 الي 4 لوغريثم علي التوالي بالمقارنة بإنخفاض 3 الي 5 لوغريثم للفيروس الذي لم يتم معاملته. تحسين الثبات الحراري يعطي اللقاح الثبات لفترة زمنية قصيرة دون التجميد مما يساعد علي الإنتاج، النقل والتخزين وبالتالي المساهمة في السيطرة علي وباء جدري الإبل.