THERMOSTABILITY IMPROVEMENT OF A RECENTLY DEVELOPED LIVE ATTENUATED SUDANESE CAMELPOX VACCINE

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المستخلص

تم تعريض لقاح جدري الابل السوداني المضعف المقترح والمنتج حديثا الي تسخين تدريجي لتحسين درجة ثباته الحراري .اختبار الثبات الحراري لفيروس الفاكسين عند درجة حرارة 56أظهر انخفاض معتبر وسريع في المعيار بما يعادل لو٣ الي لو٥ عند التحضين لمدة 30 و 60 دقيقه علي التوالي.

تم تعريض فيروس الفاكسين الي درجة حرارة 56 درجه مئوية لازمان متزايده وتم اخضاع التمريرة الاخيرة الي إختبار الثبات الحراري.

اعادة اختبار الثبات الحراري للفيريونات المنتقاه لفيروس الفاكسين في درجة حرارة 56 درجة مئوية لمدة 30 و 60 دقيقة اوضحت ان هنالك انخفاض في المعيار بما يعادل لو٢ الي لو٤ علي التوالي. مقارنة مع اللقاح الاصل فإن هنالك درجة من الثبات الحراري تم احرازها.

المعلومات التي جمعت في هذا العمل توضح أن اللقاح المضعف المقترح المعالج بالحرارة يمكن أن يكون لديه درجة ثبات حراري قصير دون الحوجة الي درجة حرارة التجميد اللازمة للحفظ من أجل للاحتفاظ بالفعالية المطلوبة أثناء التصنيع,النقل والحفظ. تحسين الثبات الحراري سوف يحسن من استخدام الفاكسين في الحقل لضبط انتشارمرض جدري الابل.

Abstract

A sudanese camel pox (CMP) candidate vaccine recently developed was subjected to gradual heating to improve its thermostability. Thermostability testing of the vaccine revealed that at 56˚C all the formulations lost their titer quickly with more than 3 to 5 log disappeared only in 30 and 60 minutes of incubation, respectively. The virus was then subjected to - heating at 56˚C for increasing time periods; the final passage was then retested for thermostability. Thermostability retesting of the recovered virions revealed that at 56˚C wild type virus and the attenuated candidate vaccines lost a titer of - and - log10 CCID50 upon incubation for 30 and 60 minutes, respectively.Compared with the original vaccine virus, a degree of thermostability improvement of the candidate vaccinewas attained. The information gathered in this work showed that; it is possible for the treated CP vaccine to have adequate short-term stability at non-freezing temperatures to support manufacturing, short-term shipping and storage. Improving thermostability should significantly enhance the utility of the vaccine during the field work for the control of CMP outbreaks.

Key words: camelpox; thermostability; vaccine

Introduction

Camelpox (CMP) is a species specific contagious viral disease of camels casued by an orthopoxvirus (OPV) (Moss., 2001) and characterized by localized and generalized pox lesions (Wernery and Kaaden., 1995). It appears in the areas where camel breed in Africa, north of the equator, the Middle East and Asia, it causes significant economic impact through the loss of production and sometimes death (OIE., 2008). In the Sudan CMP is considered one of the most infectious and contagious diseases that is well known and feared disease by herdsmen (Khalafalla *et al.*, 1998). For the control of CMP, some attenuated live cell culture vaccines were produced. The first report of a CP vaccine was in Soviet Union (Borisovisch., 1974). Reports of live vaccines have come from Saudi Arabia, UAE (Wernery and Kaaden, 2002) and recently from Sudan (Abd-Ellatif., 2010)

The virus of the vaccine that developed in the Sudan was found to be thermolabile (Abd-Ellatif., 2010), which considered to be a serious drawback for the efficient use of the vaccine in the field, where endemic areas have hot climate. In addition; these regions usually have poor infrastructures, the thing that make it difficult to sustain a cold chain to ensure the maintenance of vaccine potency.

The aim of this study was to improve the thermostability of this recently developed live attenuated camel pox vaccine to ensure that the required potency during distribution with minimal requirements for refrigeration during transport to villages is reserved.

Materials and Methods

Camelpox vaccine:

The CMP virus used in the present study is a locally developed vaccine candidate (Abd-Ellatif, 2010) which was developed from a pathogenic field strain that was isolated from affected camels during a field outbreak in *Butana* area, eastern Sudan (Khalafalla *et al*., 1998).

Virus propagation:

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% CO2 in air with Glasgow Modified Eagles Medium (GMEM) supplemented with 2ml glutamine.

The cell culture flasks were incubated at 37˚C in a humidified atmosphere of 5% CO2 in air. When the cells attained 90 – 100% confluence the medium was replaced with GMEM supplemented with 2% (v/v) FBS. A freeze - dried ampoule of wild type CMPV (CMPV/WT) and the attenuated vaccine candidate were reconstituted with one ml sterile DDW and subjected to bacterial, mycoplasma and fungal sterility in Thioglycolate broth, Mycoplasma agar base enriched with supplements and Sabouraud's agar respectively. A volume of 0.1 ml of the virus was inoculated onto a confluent 25cm2

cell culture flasks. The cultures were checked daily for development of cytopathic effect (CPE) and the virus was harvested when the CPE attained 70 – 80%. All the cells and medium were harvested, freeze-thawed twice to release the cell associated virus and the virus suspension was clarified by low speed centrifugation (1500 ×g for 10 min at 4˚C), aliquoted and stored at -20˚C until used.

Titration of virus infectivity:

Determination of the cell culture infective dose 50% end point ($CCID_{50}$ was done according to the method described by Villegas and Purchase. (1983). of the virus was then tested by taking a 0.5ml of the vaccine stock and heating it at 56 ˚C for 30 and 60 minutes and then they were titrated for virus infectivity.

Improving the thermostability:

A portion (0.2ml) of the vaccine stock was subjected to heating at 56 ˚C at increasing time of 10, 15, 20, 25, 30, 35, 40, 45, and 50 minutes; after each step viruses were recovered and then propagated in 25-cm^2 tissue culture flasks. The flasks were then incubated at 37˚C and examined daily with an inverted microscope. When the CPE was 70 – 90 %, the infected cultures were harvested and clarified as described previously. The supernatant was used as an inocula for the second passage.

Thermostability determination:

This final supernatant of the preparations incubated at 50 °C was harvested and its titer (CCID 50%) determined as mentioned before**.**

Result

Freedom from bacterial and fungi contamination was confirmed by the absence of any growth on selective media.

Thermostability testing:

Untreated vaccine:

The virus in the candidate vaccine preparation was titrated in Vero cells. The cell culture infective $dose_{50}$% ($CCID_{50}$/ml) was found to be $10^{5.5}$ $TCID_{50}$/ml.

Treated vaccine for 30 mins:

Camelpox vaccine virus was heated at 56Cº for 30 minutes and titrated, the tissue culture infective $dose_{50}$% was found to be $10^{2.6}$ $TCID_{50}$/ml.

Treated vaccine for 60 mins:

Camelpox vaccine virus was heated at 56Cº for 60 minutes and titrated, the tissue culture infective dose$_{50}$% was found to be $10^{0.8}$ TCID$_{50}$/ml.

Improving thermostability:

Thermostability retesting:

Untreated passaged vaccine:

The final passage of the live attenuated vaccine was titrated. The tissue culture infective dose$_{50}$% was found to be $10^{6.5}$ TCID$_{50}$/ml.

Treated passaged vaccine for 30 mins:

The final passage of the live attenuated vaccine was heated at 56Cº for 30 minutes and titrated. The tissue culture infective dose$_{50}$% was found to be $10^{4.5}$ TCID$_{50}$/ml.

Treated passaged vaccine for 60 mins:

The final passage of the live attenuated vaccine was heated at 56Cº for 60 minutes and titrated. The tissue culture infective dose$_{50}$% (TCID$_{50}$/ml) was found to be $10^{2.8}$ TCID$_{50}$/ml.

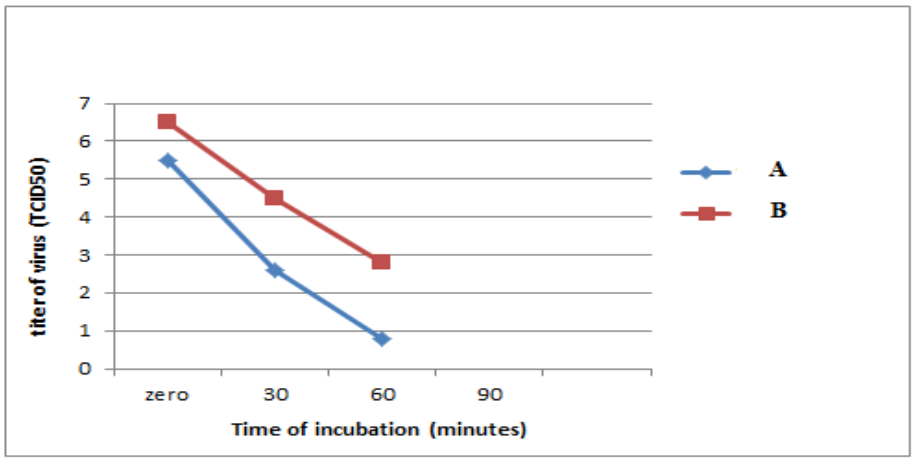

Figure 1: Thermostability testing of the original (A) and the treated vaccine virus (B) at 56Cº for 30 and 60mins.

Discussion

Camelpox is a wide-spread infectious viral disease of Old World camelids. New World camelids are also susceptible. The disease occurs throughout the camel-breeding areas of Africa, north of the equator, the Middle East and Asia, causing economic impact through loss of production and sometimes death (OIE, 2008). In the Sudan it is considered as one of the most infectious and contagious disease that has been diagnosed due to high morbidity (9%), mortality (1.2%) and a case fatality rate of 14%, loss of condition and weight in all stages as well as a reduction in milk production (Khalafalla, 1998). To herdsmen, camelpox is a well known and feared disease; serological survey demonstrated that CPV is widely spread in all parts of the Sudan where camels are raised (Khalafalla *et al.*, 1998). Effective control programs such as sanitary measures, quarantine of infected areas, restriction of camel movements, management of drinking water and a voidance of skin abrasions unfortunately are difficult to apply owing to the migratory pattern of camel production in Sudan in addition to difficulty to reach camels especially during the rainy season (Khalafalla *et al*., 1998).

The first availability of CP vaccine was reported in the 1973 in the Soviet Union. However, that report lacks data concerning the nature of the vaccine (Borisovisch, 1974). Reports of live attenuated vaccines against CP have come from Saudi Arabia, UAE (Wernery and Kaaden., 2002). Local live attenuated vaccine was recently developed from circulating virus and it was found to be relatively thermolabile (Abd Ellatif., 2010). All conventional live vaccines have the disadvantage of that they 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 nomadic system. This study was conducted to improve thermostability of the developed vaccine to retain the required potency during distribution with a minimal requirement for refrigeration during transport to villages insuring that at least the minimum immunogenic titer is delivered.

Thermostability of the candidate vaccine was tested. A series of selection of relatively thermostable progeny viruses was done. The vaccine stock was subjected to heating at 56 $^{\circ}$C at increasing time (10, 15, 20, 25, 30,35, 40, 45, and 50 minutes), and recovered viruses at each step were propagated in 25-cm^2 tissue culture flasks. Recovered viruses

from the last passage were propagated and tested for thermostability. Rapid loss of titer were obtained concerning thermostability of the candidate vaccine Abd Ellatif. (2010). According to the results of the present study it can be concluded that a degree of 10 log 1 $TCID_{50}$/ml. 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 CP vaccine since higher virus titers with 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; nevertheless the results presented herein constitute valuable information to substantially improve the CP vaccine production and stability.

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