Biologicals 40 (2012) 495–498

Contents lists available at SciVerse ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

A case of mistaken identity? Vaccinia virus in a live camelpox vaccine

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ARTICLE INFO

Article history:
Received 18 October 2011
Received in revised form
12 March 2012
Accepted 10 May 2012

Keywords:
Orthopox
Camelpox
Vaccinia
Lister
Sequencing
Contamination

ABSTRACT

Live-attenuated (LA), and inactivated adjuvant (IA) camelpox virus (CMLV) vaccines are produced in several countries worldwide. A tissue culture attenuated CMLV isolated (Jouf-78) is used to produce an LA vaccine in Saudi Arabia (Hafez et al., 1992). DNA extracts from the Saudi LA vaccine were used as positive controls for a routine ATIP PCR produced fragments longer than 881 bp. PCR-amplified ATIP sequences were similar to vaccinia virus (VACV) Lister strain. PCR and sequence analysis of two extracellular enveloped virus (EEV)-specific (A33R and B5R), and two intracellular mature virus (IMV) (L1R and A27L) othrologue genes from the vaccine DNA extracts confirmed the finding. CMLV sequences were not detected in vaccine DNA extracts. A VACV Lister strain imported from Switzerland was used in control experiments during initial testing of the Saudi LA vaccine. High antigenic similarity between VACV and CMLV, and a possible contamination event during production may have caused this issue. Environmental and health impact studies were recommended because early VACV vaccines produced in some European countries contained nonhighly attenuated strains that were not adequately screened for adventitious agents.

1. Introduction

Camelpox is caused by a poxvirus belonging to Family *Poxviridae*, subfamily *Chordopoxvirinae*, genus *Orthopoxvirus*. Viruses belonging to genus *Orthopoxvirus* (OPV) are antigenically closely related. Members/species include *Variola major virus* (VARV), *Monkeypox virus* (MPV), *Camelpox virus* (CMLV), *Vaccinia virus* (VACV), *cowpox virus* (CPV), and six other species not known to be pathogenic for humans [1,2]. The poxvirus genome is a double-stranded DNA molecule of 130–375 kbp. The central part of the genome is highly conserved in gene order, content and sequences whereas the terminal regions vary both in length and patterns of restriction enzyme cleavage sites [2].

Laboratory diagnosis of CMLV include TEM, demonstration of CMLV antigens in tissues, and scabs by immunohistochemistry, isolation on the chorioallantoic membrane (CAM) of embryonating chicken eggs (ECE) and, detection of viral nucleic acid using PCR and restriction analysis [3]. PCR, real-time PCR, and sequencing are the methods of choice for rapid identification and differentiation of poxviruses up to the species level [4,5].

Live-attenuated (LA), and inactivated adjuvant (IA) camelpox virus (CMLV) vaccines are produced in several countries worldwide [3]. In-process, and final quality control (QC) procedures adopted by production plants must ensure seed virus purity and identity. An absolutely fail-safe system that would result in no risk of releasing an unsatisfactory product would probably be too expensive with regard to cost of production as well as control [6]. Here, we report finding a VACV in a commercial vaccine that should include a tissue culture attenuated CMLV, and we discuss the possible consequences of such a finding.

2. Materials and methods

2.1. CMLV vaccine samples

Two vials of a lyophilized LA virus vaccine for camelpox were kindly provided by the Ministry of Agriculture, Saudi Arabia in 2009. The vials were labeled "Veterinary Vaccine Production Center (V.V.P.C.) Modified live virus vaccine for camelpox, Batch #01004, Shelf life: 30/3/2012, contains 25 doses". The vials were kept at −20 °C in the Central Biotechnology Laboratory, College of

Abbreviations: LA, live attenuated; IA, inactivated adjuvant; CMLV, camelpox virus; VACV, vaccinia virus; EEV, extracellular enveloped virus; IMV, intracellular mature virus; TEM, transmission electron microscopy; CAM, chorioallantoic membrane; ECE, embryonating chicken eggs; QC, quality control.

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doi:10.1016/j.biologicals.2012.05.001

Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa. Vials were opened in a class II BSC, and re-hydrated using 1 ml nuclease-free sterile distilled water. Vials were kept on ice until aliquots were aspirated using nuclease-free filtered tips.

2.2. Viruses

A CMLV isolated on specific pathogen-free (SPF) embryonating chicken eggs (ECE) (Al-Ahsa 2009) was used as a positive control in all PCR reactions. Al-Ahsa 2009 CMLV identity was previously confirmed using ATIP PCR and sequencing [5]. A commercial capripox virus vaccine was used as a negative control in ATIP PCR reactions. The vaccine was kindly provided by the Ministry of Agriculture, Saudi Arabia.

2.3. Total DNA extraction using commercial silica-based spin columns

Qiagen Dneasy Blood & Tissue Kit (QIAGEN Sciences, Maryland, USA) was used for the extraction of total DNA from vaccine and control samples. Extractions were performed according to the manufacturer's recommendations using 50 μl of homogenized CAM with CMLV pock lesions, 50 μl of reconstituted camelpox vaccine, and 50 μl of reconstituted capripox vaccine as starting materials. DNA extracts were kept frozen at −20 °C until tested using PCR.

2.4. Oligonucleotides

Primers that amplify a region of the OPV Acidophilic-Type Inclusion Protein (ATIP) gene were described before [7]. Two extracellular enveloped virus (EEV)-specific othrologue genes (A33R and B5R) were also targeted for amplification. The primers used were A33R upper (5′-GGCATATGATGACACCAGAAAACG-3′), A33R lower (5′-GGCTCGAGT TAGTTCATTGTT TTA ACA C-3′), B5R upper (5′-GGC ATA TGA AAA CGA TTT CCG TTG TTA CG-3′), and B5R lower (5′-GGC TCG AGT TAC GGT AGC AAT TTA TGG-3′) [8]. In addition, two intracellular mature virus (IMV) genes (L1R and A27L) were also targeted for amplification. The primers used were L1R upper (5′-GGC ATA TGG GTG CCG CAG CAA GC-3′), L1R lower (5′-GGC TCG AGT CAG TTT TGC ATA TCC G-3′), A27L upper (5′-GGC CAT GGA CGG AAC TCT TTT CCC CG-3′), and A27L lower (5′- GGC TCG AGC TCA TAT GGA CGC CGT CC-3′) [8].

2.5. PCR

ATIP, A33R, B5R, L1R and A27L amplification reactions were carried out using the HotStar Taq® Plus Master Mix PCR kit (QIAGEN, Hilden, Germany). Total DNA extracts from vaccines, and CMLV control were tested using the same master mix. Each reaction tube contained 1 unit of HotStar Taq® Plus DNA polymerase in 1× PCR buffer containing (KCl and $(NH_4)_2SO_4$), 200 μM of each dNTP, 0.5 μM of each of the forward and reverse primers, 1× CoralLoad dye mix, and 3 μl of a 1:50 dilution of each of the tested vaccine extracts, and controls. The reaction tubes were centrifuged briefly, and placed in a BioRad MyCycler™ thermal cycler (BioRad, Hercules, California, USA). ATIP PCR reactions conditions were described previously (Meyer et al., 1994). Thermal cycling conditions for amplification of the A33R and B5R ortholog genes was also described previously [5]. Thermal cycling conditions for amplification of the L1R ortholog gene were: an initial denaturation step (95 °C for 4 min), 15 first-stage amplification cycles (95 °C for 1 min, 60 °C for 1 min, and 72 °C for 2.5 min), 25 s stage amplification cycles (95 °C for 30 s, 70 °C for 30 s, and 72 °C for 2.5 min), and a final extension step (72 °C for 5 min). Thermal cycling conditions for amplification of the A27L ortholog gene were: an initial denaturation step (95 °C for 4 min), 15 first-stage amplification cycles (95 °C for 1 min, 50 °C for 1 min, and 72 °C for 2.5 min), 25 s stage amplification cycles (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 2.5 min), and a final extension step (72 °C for 5 min). After amplification, 15 μl were analyzed by electrophoresis on a 1.2% agarose gel stained with ethidium bromide. DNA bands were visualized by UV irradiation in a Gel Doc XR gel documentation system (BIO-RAD laboratories, Milan, Italy).

2.6. Sequencing

PCR products were purified from gels using QIAEX® Gel Extraction Kit (QIAGEN Sciences, Maryland, USA). Purified PCR products were sequenced in both orientations by the dideoxy chain-termination method using the amplification primers described above. The nucleotide sequences of these genes were aligned using CLUSTAL W [9], and BLASTN 2.2.25 search program (National center for Biotechnology Information (NCBI) [10].

3. Results and discussion

3.1. Saudi LA vaccine produces ATIP PCR amplicons longer than the 881 bp

The fastest and simplest methods of routine confirmatory diagnosis of CMLV infections rely on PCR-based-assays to avoid the known high degree of antigenic cross-reactivity between members of the genus *Orthopoxvirus* [11]. PCR assays require the use of positive and negative controls. Lyophilized commercial vaccines are ideal PCR control candidates when proper extraction and quantification technologies are applied. DNA extracts from the Saudi LA vaccine were used as positive controls during the investigation of an outbreak of camelpox in the Eastern Saudi Arabia [5]. Unexpectedly, ATIP PCR using DNA extracted from the camelpox vaccine produced fragments about 1569 bp (Fig. 1). ATIP PCR amplicons longer than the 881 bp characteristic for CMLV have been reported for VACV WR strain [7]. To confirm the Saudi LA vaccine virus identity, PCR-amplified ATIP sequences were sequenced (GenBank Acc # JF812341). BLAST comparisons of a 228 bp fragment of the Saudi LA vaccine ATIP sequences revealed 99% identity to VACV Strain Lister (GenBank ID: AY678276.1) and the clone VACV107

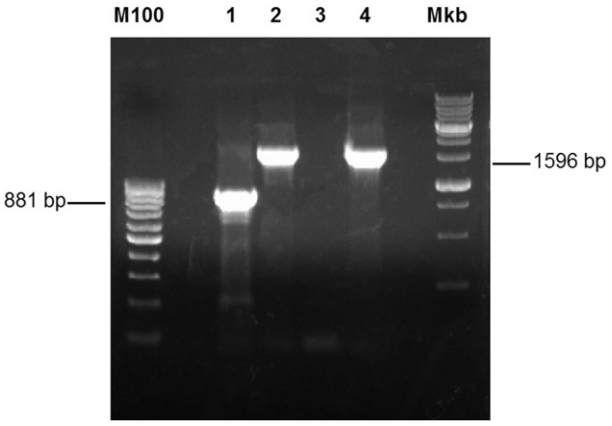

Fig. 1. ATIP gene PCR product analysis. ATIP primers (Meyer et al., 1994) were used to amplify OPV sequences from 1:50 dilutions of DNA extracts from the Saudi camelpox vaccine and controls. M100: 100 bp DNA ladder. Mkb: 1 kb DNA ladder. Lane 1: ATIP PCR product for control CMLV. Lanes 2 and 4: ATIP PCR product for two independent vials from batch # 01004 of the Saudi camelpox vaccine. Lane 3: ATIP PCR for a control commercial capripox virus vaccine. ATIP gene amplicons were larger than the 881 bp characteristic of CMLV and similar to reported VACV sequences.