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Detection and isolation of Bluetongue virus from commercial vaccine batches

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ABSTRACT

In this report we describe the detection and identification of Bluetongue virus (BTV) contaminations in commercial vaccines. BTV RNA was detected in vaccine batches of Lumpy skin disease (LSD) and Sheep pox (SP) using quantitative PCR (qPCR) for VP1 and NS3 genes. Both batches were positive for VP1 and NS3 in qPCR. The LSD vaccine-derived sample was positive for VP1 and VP2 in conventional PCR. The SP vaccine-derived sample was examined by amplification of VP1, VP4, VP6, VP7, NS2 and NS3 gene segments in conventional PCR. The SP vaccine-derived sample was further propagated in embryonated chicken eggs (ECE) and Vero cells. Preliminary sequence analysis showed that the LSD vaccine-derived sequence was 98–99% similar to BTV9. Analysis of the six genomic segments from the SP vaccine-derived isolate showed the highest similarity to BTV26 (66.3–97.8%). These findings are particularly important due to the effect of BTV on cattle and sheep, for which the vaccines are intended. They also demonstrate the necessity of rigorous vaccine inspection and strict vaccine production control.

1. Introduction

Q4 Contamination of vaccines and other biological products intended for medical and veterinary use is a major concern in the bio-products industry [1,2]. Detection of vaccine contaminants was reported to be performed by fluorescent antibody test [3], Western analysis [4], electron microscopy, cytopathic effect assay (CPE) [5], reverse transcriptase (RT) activity test for detection of retroviruses [6], and deep sequencing [2]. Recently, porcine circovirus1 (PCV1) was discovered in commercial vaccine batches of human rotavirus [7]. This finding led to a temporary halt on the use of this vaccine in the USA [8], to a subsequent update in the regulations of manufacturing procedure (European Medicines Agency), and to an effort to improve the detection limits of low level viral contamination [9]. This case may therefore highlight the significance of vaccine safety and the potential implications of vaccine contamination. Other reports on viral contaminations of commercial vaccine described Simian monkey viruses in polio vaccines [10] and Bluetongue virus contamination in canine distemper vaccine [11]. Importantly, attenuated vaccines, prepared with primary cell cultures, are prone to unintentional viral and bacterial contaminations that can be transferred to the vaccine recipients [4]. Typical examples are mycoplasma (http://www.vaclib.org/sites/vac_coming_thru.html) and bovine viral diarrhea virus (BDV) (http://www.nybloodcenter.org/PatentsAndLicensing/SDTechnology.html). BVD is a potential contaminant of commercial fetal bovine serum (FCS) that is used for cell growing in vaccine preparation, and may therefore contaminate cell culture-based vaccines.

Bluetongue virus (BTV) is a ds(-)RNA non-enveloped arbovirus, a member of the *Reoviridae* family, genus *Orbivirus* [12]. The disease caused by this virus affects domestic and wild ruminants, leading in many cases to severe illness and death [13]. BTV disease has been diagnosed in Australia, North America, Africa, the Middle East, Asia and Europe. Recent Bluetongue outbreaks in Europe have led to major economic losses, with a partial estimation of over 2 billion Euros [13,14]. The fast rate by which changes occur in the gene encoding VP2, and to a lesser extent, VP5, has led to over 25 different serotypes [15]. This dynamic state can potentially lead to generation of new, putative more virulent variants of the virus. Therefore, the introduction of such a pathogen via the dissemination of a contaminated vaccine, poses a potentially severe threat to domesticated animals. In this report we describe the identification and subsequent isolation of BTV from commercial Sheep pox (SP) vaccine, and identification of BTV presence in commercial Lumpy skin disease (LSD) vaccine.

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2. Materials and methods

2.1. Vaccine origin

Attenuated (live) Sheep pox vaccine (SP) and Lumpy skin disease vaccine (LSD) batches were purchased from two international manufacturers. Vaccine batches underwent routine inspection for the presence of contaminant viruses or bacteria (BTV, BVDV, Herpes viruses, Mycoplasma, etc.) prior to authorizing the release of the batches for use in Israel.

2.2. Viral RNA isolation

Vials from each batch were randomly selected for RT-qPCR. Isolation of viral RNA from SP (a total of 5 vials) and LSD (a total of 4 vials) vaccines was done by re-suspending the lyophilized powder in 1 ml PBS, pH 7.2. RNA from re-suspended vaccine and suspension frominfected Embryonated chicken eggs (ECE) and cultured cells was extracted using the INVISORB® spin virus RNA mini kit from Stratec (http://www.stratec.com/en/molecular/) according to the manufacturer's instructions. RNA samples that were positive in the commercial pan-BTV NS3 real-time PCR assay (VetMax™, France) were used for further examination. Non-contaminated samples and RNA/DNA free water were used as controls for the RNA extraction procedure and PCR assays.

2.3. Real-time PCR

RT-qPCR for VP1 gene for vaccine re-suspension, ECE and Vero cells RNA extractions was performed either as described by Shaw et al. [16], or using the Bluetongue virus NS3-All Genotypes kit from LSI (VetMax™, France), according to the manufacturer's instructions. The extracted RNA was denatured at 98 °C for 3 min and placed immediately on ice prior to the reaction setup. All injected embryos from both batches of vaccines that died were tested with the commercial qPCR. Infected Vero cells that showed Cytopathic effect (CPE) upon infection with ECE homogenates previously found positive in commercial qPCR were consequently tested by the commercial qPCR protocol. As a positive control, RNA from BTV4 (Israeli isolate) was used. In order to exclude the possibility of laboratory contamination, three negative controls were used for the qPCR: (1) template RNA from non-contaminated batch, (2) no-RNA sample (ddH_2O) for the RNA extraction, and (3) no template control for the qPCR procedure.

2.4. Conventional PCR

Positive samples from the Real-time PCR test were further examined using conventional PCR and the amplified products were gel-purified and sequenced. The VP1 gene segments from the SP vaccine-derived RNA were amplified using three overlapping primer sets, to amplify a total of 1931 bp region. For the LSD vaccine-derived RNA, two primer sets were used for two non-overlapping regions: bases 1 to 434 and 1353 to 1942 of segment 1.

Regions of segments 4 (VP4), 7 (VP7), 8 (NS2), 9 (VP6) and 10 (NS3) were amplified using single primer sets. Except for primer ORBI-UNI-F and R, which was designed by Shaw et al. [16], all primers were designed for this study. All primers and expected product sizes are detailed in Table 1. For amplification of segments 1 (VP1), 7 (VP7) and 10 (VP3) regions, the OneStep RT-PCR kit (QIAGEN, Germany) was used according to the manufacturer's protocol. The reaction setup was as follows: 5 μl Buffer, 1 μl dNTP, 1 μl Enzyme, final concentration of 15 pmol/μl of each primer, 11 μl RNA/DNA-free water and 5 μl heat-denatured RNA. The conditions for these reactions were as follows: 30 min at 45 °C, 10 min at 95 °C, 40 cycles of: 30 s at 94 °C, 1 min, at 54 °C, 2 min at 68 °C, and final elongation of 5 min at 68 °C. Regions from segments 4 (VP4), 8(NS2) and 9(VP6) were amplified using the Maxime RT-PCR mix (iNTRON, http://www.intronbio.com/) according to the manufacturer's instructions. The amplification conditions for NS2 and VP6 were: 30 min at 45 °C, 5 min at 94 °C, 35 cycles of: 30 s at 94 °C, 30 s at 53 °C, 1.2 min at 72 °C, and final elongation step of 5 min at 72 °C. The conditions for the VP4 amplification were identical with the exception of 2.5 min extension time instead of 1.2 min the following control reactions were run for the conventional PCR: (1) non-contaminated batch template RNA and (2) no-template control for the PCR.

The following GenBank accession numbers were assigned for the new sequences obtained in this study: LSDV vaccine-derived: VP1 – KT946755, KU710719; VP2 – KU710717.

Table 1
Primers used for conventional PCR. The following primers were used to characterize the identified BTV contaminants by conventional PCR. The name, origin and expected product size for each primer set are indicated.

Target	Primer name	Primer sequence (5′ to 3′)	Template	Product size (bp)
VP1	UNI-VP1-1F (F)	GTTAAAATGCAATGGTCGCAAT	SP + LSD vaccines	
VP1	UNI-VP1-810-R (R)	CCCCACATCTTYACAAACCA	SP vaccine	829
VP1	ORBI-UNI-R (R)[a]	TGCATYTCGTTTTTMGC	LSD vaccine	430
VP1	26VP1-713F (F)	CAC TAA GGT TGA CCC CAT AGG	SP vaccine	
VP1	26VP1-1432R (R)	AA GCT TTA ATC CGT GAT GTG A	SP vaccine	742
VP1	UN-VP1-1353-F (F)	ACAAGTTCRGGYTTTTCAAC	SP + LSD vaccines	
VP1	UN-VP1-1920-R (R)	YCYTCTCCATATCCAAAATCTAT	SP + LSD vaccines	590
NS3	UNI-NS3-1F (F)	GTTAAAAAGTGTCGCTGCCA	SP vaccine	
NS3	UNI-NS3-781R (R)	CCCTCCCCCGTTAGACAGCAG	SP vaccine	779
VP7	VP7-select-F (F)	GTTAAAAATCTTTAGAGATGGACACTAT	SP vaccine	
VP7	VP7-select-R (R)	CCGTGCAAAGTGGACTACACAT	SP vaccine	1081
VP6	BTV Seg9 31 FWD (F)	CTACTTGCACCCGGTGACGTGAT	SP vaccine	
VP6	BTV seg9 1029 REV (R)	CCCTGGACCCTTTAGAGGTGATC	SP vaccine	998
NS2	BTV Seg81 FWD (F)	GTTAAAAAATCCTTGAGTCATGGAGC	SP vaccine	
NS2	BTV seg8 1118 REV (R)	GTAAGTGTAAAATCCCCCCC	SP vaccine	1118
VP6	BTV seg4 1 FWD (F)	GTTAAAACATGCCTGAGCCACACGCAG	SP vaccine	
VP6	BTV seg 4 1948 REV (R)	TGCCCCCCTCACCTAGCAGTCACGC	SP vaccine	1948
VP2	BT-5+9-VP2-F (F)	TATGCRTTGCCAATYAGYTT	LSD vaccine	
VP2	BT-5+9-VP2-R (R)	TGTAWGCTCYAGGAGTCYCA	LSD vaccine	340

(F), Forward primer.
(R), Reverse primer.
[a] This primer was designed by Shaw et al. [16].

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