Original article

Retrospective genome analysis of a live vaccine strain of bovine viral diarrhea virus

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Abstract – A live bovine viral diarrhea (BVDV) vaccine, marketed as a derivate of the Oregon C24V strain, was used between the end of the 1960s and the beginning of the 1990s in Central Europe. Since laboratory investigations of mucosal disease cases in vaccinated animals suggested recombinations between the vaccine and wild type variants of BVDV, and recombinational nucleotide sequences seemed distinct from BVDV Oregon C24V, the aim of the present retrospective study was to analyze the genomes of pre-registration (termed here BVDV-Xpre) and of marketed (BVDV-X) batches of the vaccine. The results of the complete genome analysis of BVDV-Xpre confirmed that the original virus strain used at the start of the vaccine production was Oregon C24V. Surprisingly, the analysis of the complete nucleotide sequence of the BVDV-X marketed vaccine revealed that this strain belongs to the BVDV 1b subgroup, with a 93.7% nucleotide sequence homology to BVDV reference strain Osloss. The homology to BVDV Oregon C24V was significantly lower (77.4%), and a thorough sequence scanning showed that the genome of BVDV-X had not derived from Oregon C24V. These data indicate the very likely scenario that a strain different to Oregon C24V was picked up during the in vitro or in vivo passages for vaccine development. Despite of the virus-switch, the BVDV-X vaccine continuously maintained its innocuity and efficacy, as proven by the regular quality testing data, and the presence of the foreign virus remained unnoticed over many years. The results of this work emphasize that the contamination of commercially available live vaccines with exogenous BVDV strains is a real risk factor, and a unequivocal analysis, including molecular methods, is needed to verify their authenticity.

bovine viral diarrhea virus / vaccine / strain switch / control

1. INTRODUCTION

Bovine viral diarrhea virus (BVDV), a major pathogen of cattle, is a member of the *Pestivirus* genus that together with the *Flavivirus* and *Hepacivirus* genera belongs to the Flaviviridae family [39, 41]. BVDV is an enveloped virus with an uncapped and non-polyadenylated positive-stranded RNA genome of about 12.5 kb in size. The genome comprises a single open reading frame (ORF) that is flanked by 5' and 3' untranslated regions (UTRs) [11, 12, 14]. The 5'UTR function is a so-called internal ribosome entry site (IRES) that promotes cap-independent translation initiation [30, 31]. The

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ORF encodes a polyprotein of about 4 000 amino acids that is co- and post-translationally processed by viral and cellular proteases into 11 or 12 mature viral proteins: NH_2-N^{pro}, C, E^{rns}, E1, E2, p7, NS2-3, NS4A, NS4B, NS5A, NS5B-COOH [34].

According to their ability to cause a cytopathic effect (CPE) in cell cultures, BVDV strains are differentiated as cytopathogenic (cp) or noncytopathogenic (ncp) biotypes [18, 22]. Both cp and ncp biotypes of BVDV are involved in the pathogenesis of mucosal disease (MD), a fatal clinical manifestation of BVDV (reviewed in [23]). Infection of pregnant cattle with ncp BVDV in the first trimester of gestation results in the birth of persistently infected (PI) calves. PI animals are immunotolerant against the respective ncp virus. After superinfection with an antigenically closely related cp strain, which is either of exogenous origin or develops by genetic alterations of the resident ncp virus, the PI animals are predisposed to develop early onset of MD [6, 8], whereas superinfection of PI animals with an antigenically different cp BVDV can result in the late onset of MD [16, 35].

A cp and an ncp BVDV strain, isolated from the same animal succumbing to MD, are termed a "virus pair". Molecular analysis of different BVDV pairs indicate that cp BVDV strains evolve in vivo from ncp BVDV by RNA recombination in cattle persistently infected with ncp BVDV (reviewed in [23, 38]). These recombination events result in insertions of cellular sequences, duplications, rearrangements or deletions of viral sequences, affecting the NS2-3 region [2, 4, 21, 23] or the N-terminal region of the BVDV genome [24, 25]. These changes in the genomes of cp BVDV strains lead to the expression of non-structural protein NS3, which is considered the marker protein for cytopathogenicity. However, mutations in the NS4B were shown to abolish cytopathogenicity despite NS3 production [33]. Interestingly, some cp BVDV strains show no recombination-induced genome alterations [20, 29, 32].

To protect cattle against the various forms of BVDV infections, both modified live and inactivated vaccines are used. A live vaccine, termed here for ethical reasons as BVDV-X, was used in Central Europe from the end of the 1960s to the beginning of the 1990s. The vaccine was prepared from a seed stock of the cp BVDV reference strain Oregon C24V that was serially passaged in cell cultures and in animals. After registration, the vaccine has undergone regular quality testing. During the use of the vaccine for more than two decades, the following main observations were made: (i) vaccination provides sufficient protection against postnatal infections; (ii) from field observations and laboratory diagnosis, it is suspected that vaccination of PI animals might have led to the development of an early onset of MD (Pálfi, personal observation).

In a recent study, four cp viruses originating from these MD cases were investigated [1]. Nucleotide sequence analysis of the complete NS2-3 region of the cp field isolates and the BVDV-X vaccine strain confirmed the theory of early onset of MD in vaccinated animals. Surprisingly, the sequence of the NS2-3 gene of the vaccine virus was found to be different from that of the Oregon C24V strain, and included a viral insertion located at the same position as the insertion found in BVDV strain CP7 [37].

To clarify the identity and the origin of the BVDV-X strain, three vaccine batches (one prior to and two after the registration process) were obtained and examined. Analysis of the 5'UTR of an original batch prior to registration (named here as BVDV-Xpre), proved that this genomic region of the master stock was identical with BVDV Oregon C24V. However both batches of the virus from the marketed vaccine had Osloss-like 5'UTR sequences. To further elucidate the questions raised, the complete nucleotide sequences of the pre-registration batch (BVDV-Xpre) and of one of the marketed vaccine batches (termed BVDV-X) were determined and compared with published full-length sequences of BVDV strains,

including Oregon C24V. Since at the time of registration of BVDV-X the methods of identifying different BVDV strains were not so developed as at the present, our basic goals were the followings: (i) verifying, whether the Oregon C24V strain was the starting point of the BVDV-X vaccine production; (ii) investigating the presence and ratio of Oregon C24V nucleotide sequences in the genome of the marketed vaccine BVDV-X. Our hypothesis was that finding both Oregon C24V and Osloss-like nucleotide sequences in the different batches of the BVDV-X vaccine could reveal that recombination events occurred between the original Oregon C24V strain and a heterologous BVDV virus strains during the numerous passages in vitro and in vivo.

2. MATERIALS AND METHODS

2.1. Cells and viruses

Secondary bovine turbinate (BT) cells were cultured in Eagle Minimum Essential Medium (EMEM) supplemented with 2 mM of L-glutamine and 10% foetal calf serum (FCS). By following routine procedures used at our institute, cells and FCS were tested regularly for the absence of *pestiviruses* by a reverse transcription-PCR (RT-PCR) targeting the 5'UTR [15] and by a routine immunoperoxidase assay (IPX) using BVDV polyclonal antiserum (VLA, Addlestone, UK). For FCS, the absence of anti-*pestivirus* antibodies was shown by lack of virus neutralization following standard procedures used at our institute. The BVDV-Xpre and two different batches of the marketed vaccine originating from the 1980s and 1990s were obtained from the manufacturer.

2.2. Infection of cells

To obtain pure virus stocks, the three batches were plaque purified by standard methods. Briefly: for propagation of the viruses, 80% confluent cell monolayers in 6-well tissue culture plates were inoculated with ten-fold dilutions of the respective virus strains. After 1 h adsorption, the cells were washed with EMEM, then overlaid with low melting agarose (Sigma-Aldrich, St. Louis, MO, USA) containing EMEM and 2% FCS. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 for 72 h. Five plaques of each batch were picked and used to infect fresh BT cells. The plaques were examined by the above-described routine RT-PCR. The PCR products were sequenced and compared in order to rule out that the vaccine batches did not contain mixed virus populations. Virus particles were pelleted from the supernatants of infected BT cells by ultracentrifugation (Airfuge; Beckman Instruments, Fullerton, CA, USA), at 55 000 × g for 3 h. The virus stocks were resuspended in PBS without Ca^{2+} and Mg^{2+} and kept at –70 °C.

2.3. RNA extraction and cDNA synthesis

RNA was extracted from virus stocks using the TRIzol LS reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. To obtain long cDNA copies of the viral genomes, the Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and an antisense primer (VDAS1 located in the 3'UTR of BVDV, see Tab. I) were used. The transcription was performed in 20 µL reaction mixtures containing 500 ng RNA, 40 pmole primer, 200 µM of each dNTP, 24 U of RNAse inhibitor (Amersham Biosciences, Piscataway, NY, USA) and 200 U of Superscript II.

2.4. PCR amplification and sequencing

For the amplification of overlapping fragments of the whole viral genome, the Expand Long Template Kit (Roche Diagnostics, Basel, Switzerland) was used. The sequences and positions of oligonucleotides used in the initial PCR assays for amplification of BVDV-X are shown in Table II. For the initial PCR assays and the subsequent sequencing of the genome of

Table I. Nucleotide and deduced amino acid sequence differences between the complete genomes of Oregon C24V and BVDV-Xpre. Silent mutations are marked with dashes.

Nucleotide position	Nucleotide substitutions		Amino acid substitutions	
	Oregon C24V	BVDV-Xpre	Oregon C24V	BVDV-Xpre
2112	C	T	–	–
2283	C	T	Alanine	Valine
2533	A	G	Asparagine	Aspartic acid
3883	A	T	–	–
8835	G	A	Glycine	Glutamic acid
9411	G	A	Arginine	Histidine
9586	G	A	–	–
10372	T	A	–	–
10426	G	A	–	–
11458	C	T	–	–

Table II. List of the primers used in this study for generating initial PCR products.

Primer	Sequence (5'–3')	5' position*
13A[a]	GCT AGC CAT GCC CTT AGT AGG A	101
12A[a]	GGC CTT TGC AGC ACC CTA TCA G	325
14A[a]	ATC AAC TCC ATG TGC CAT GTA CAG C	372
16F	ACA ATG GAA CTT ACA A	1207
B7R	ACC AAC CAT GCT TGT TCC ACT	1419
C912F[b]	TTG ATA ACA GGG GTA CAA GG	2441
E100[b]	CAT ATG GTC TGC AAG GCA TAG G	3284
125AF	GAG GGG CCG GTA GAA AAG AC	3320
A[c]	GCA GAT TTT GAA GAA AGA CAC TA	4934
B[c]	TTG GTG TGT GTA AGC CCA	5318
C[c]	GTG GAG ACT GGG AAA GCA CT	7163
125BR	GCA TAY TGG AGG TGG GTK GTG T	7323
NS5BR1	AGG CTG GGT TGG GCT ATT GTG TG	10498
NS5BF3	TTG CCT ATA GGT TTG AGG ACA TAG	11382
OREGON32F	GCC TAT TGG TCC TCT CGC AG	11610
BVD3CF	GAG GAC GGC AAT TGG CTG GTA	11843
VDAS1	CTG TGT GCA TTR ART GTA GTG TT	12242

* Nucleotide position corresponding to BVDV SD-1.
[a] See in [15].
[b] VLA.
[c] See in [19].

BVDV-Xpre, a set of 32 primers designed based on the published sequence of BVDV Oregon C24V was used. The sequences of these primers are available upon request. The reactions were prepared in a 50 μL total volume, containing 1× buffer for a final MgCl$_2$ concentration of 2.25 mM, 200 μM of each dNTP, 20 pmole of each primer, 5 μL of cDNA and 2.5 U of Expand polymerase mix. After 2 min initial denaturation, 36 cycles were performed with the following parameters: 45 s at 94 °C, 45 s at the annealing temperature of the corresponding primer, 1 min/kb of the sequence at 68 °C. After cycling, a final extension was applied for 10 min at 68 °C.

PCR products were analyzed on 0.8% agarose gels. The amplicons were purified by using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The purified DNA products were sequenced from both directions first with the same primers as used for the amplification reactions. Sequencing was performed in an ABI Prism sequencer (Model 377), using the Big Dye Terminator V3.1 sequencing kit (Applied Biosystems, Foster City, CA, USA). After initial sequencing, further oligonucleotides were designed based on these data to complete the whole genomic sequence. The preliminary sequencing of the different plaques of the pre-registration batch BVDV-Xpre showed that the examined sequences of the plaques were identical. Furthermore, recent results of sequencing the whole NS2-3 gene of the BVDV-X vaccine and cp BVDV field isolates [1], and preliminary results from sequence analysis of the plaque-purified stocks of BVDV-X showed that the virus was different from Oregon C24V. Therefore, two independent cDNA originating from a single plaque of the pre-registration batch (BVDV-Xpre) as well as one batch of the marketed vaccine strain (BVDV-X) were amplified and fully sequenced. A third PCR product was generated and sequenced to determine the consensus sequence, when differences were found.

2.5. Determination of the 5' and the 3' terminal sequences

To determine the 3' end of the BVDV-Xpre genome, RNA ligation was performed with T4 RNA ligase (New England Biolabs, Beverly, MA, USA), followed by RT-PCR. Viral RNA was reverse-transcribed using M-MLV RT (Invitrogen, Carlsbad, CA, USA) and a reverse primer 14A located in the 5'UTR region. Subsequently, amplification was performed using oligo Oregon32f, located in the NS5B gene, as the upstream primer and oligo12A, located in the 5'UTR, as the reverse primer (Tab. II). The same strategy was followed for BVDV-X, using a reverse primer BVD7R located in the E^{rns} region for reverse transcription. Subsequently, amplification was carried out using oligo BVD3CF, located at the end of the NS5B gene, as the upstream primer and oligo14A, located in the 5'UTR, as the reverse primer (Tab. II). For determination of the 5' end of the two genomes, the 5'RACE Version 2.0 system (Invitrogen, Carlsbad, CA, USA) was used according to the manufacturer's instructions. The PCR products were purified and sequenced. Sequences were compared to the 5' and 3' ends of published full-length BVDV genomes in order to determine the exact terminal sequences.

2.6. Sequence analysis

The nucleotide sequences were edited and analyzed with multiple programs of the DNASTAR software package (Lasergene, Madison, WI, USA). Search for sequence similarities in GenBank were performed using the BLAST programs (The National Center for Biotechnology Information, Bethesda, MD, USA).

2.7. Comparison with full-length sequences of BVDV strains

The complete nucleotide and deduced amino acid sequence of BVDV-Xpre was compared with the published full-length sequence of Oregon C24V. The nucleotide

and deduced amino acid sequences of BVDV-X were compared with the published sequences of the complete genomes of type 1 strains Osloss, CP7, CP75A, ILLC, ILLCNCP, NADL, NADLNCP, SD-1, Oregon, Oregon C24V, and type 2 strains 1373, NY93, and 890.

3. RESULTS

3.1. Analysis of the full-length nucleotide sequence of the BVDV-Xpre genome

The BVDV-Xpre virus proved to be a derivate of the Oregon C24V strain. There were differences at 10 nucleotide positions, and only four of them caused amino acid (aa) changes (Tab. I), affecting the E1, E2 and NS5A regions. Both the 5' and 3' UTR were found to be identical to those of the published sequence of the Oregon C24V strain.

3.2. Analysis of the complete nucleotide sequence of the BVDV-X genome

The entire genomic sequence of the live cp BVDV vaccine strain BVDV-X is composed of 12 308 nucleotides. The 5'UTR is 381 nucleotides long, whereas the 3'UTR comprises 182 nucleotides. The ORF begins at position 382 and ends at position 12 126, consists of 11 745 nucleotides, which encode a polyprotein of 3 915 amino acids.

The nucleotide sequence of the 5'UTR of BVDV-X compared with that of the published sequences of BVDV 1 and BVDV 2 strains revealed nucleotide sequence identity of 83%–98% compared with the BVDV 1 strains and 65% with the BVDV 2 reference strain 890 (Tab. III). The phylogenetic tree derived from the 5'UTR of the vaccine virus and of the published sequences revealed that BVDV-X belongs to subgroup 1b of BVDV. The closest nucleotide similarity, of 98%, was found with the Osloss strain, whereas similarity with BVDV Oregon C24V was only of 83% (Fig. 1).

Table III. Nucleotide identity of the 5'UTR and 3'UTR of the BVDV-X vaccine strain and other BVDV 1 and BVDV 2 strains.

Virus strain	Nucleotide identity (%)	
	5'UTR	3'UTR
BVDV 1 Osloss	98	95
BVDV 1 CP7	90	89
BVDV 1 ILLC	92	89
BVDV 1 NADL	83	70
BVDV 1 Oregon	83	64
BVDV 2 890	65	49

The nucleotide sequence of the 3'UTR of BVDV-X compared with that of the published sequences of BVDV 1 and BVDV 2 strains revealed nucleotide sequence identity of 64%–95% compared with BVDV 1 strains and of 49% with the BVDV 2 reference strain 890 (Tab. III). The closest nucleotide similarity, of 95%, was found with the 3'UTR of the Osloss strain, whereas similarity with BVDV Oregon C24V was only 64%.

3.3. Analysis of the polyprotein of BVDV-X

The deduced amino acid sequence of the polyprotein of BVDV-X was compared with published sequences of the polyproteins of BVDV 1 and BVDV 2 (Tab. IV). The highest amino acid variability was found in the E2 region, 84–91% aa identity with BVDV 1 strains and 62% aa identity with BVDV 2 reference strain 890. The lowest amino acid variability was found in the NS3 and NS5B regions, 94–98% and 84–97% aa identity with BVDV 1 strains and 91% and 79% aa identity with BVDV 2 reference strain 890.

3.4. Complete sequence scanning of BVDV-X to determine sequence similarity with BVDV Oregon C24V

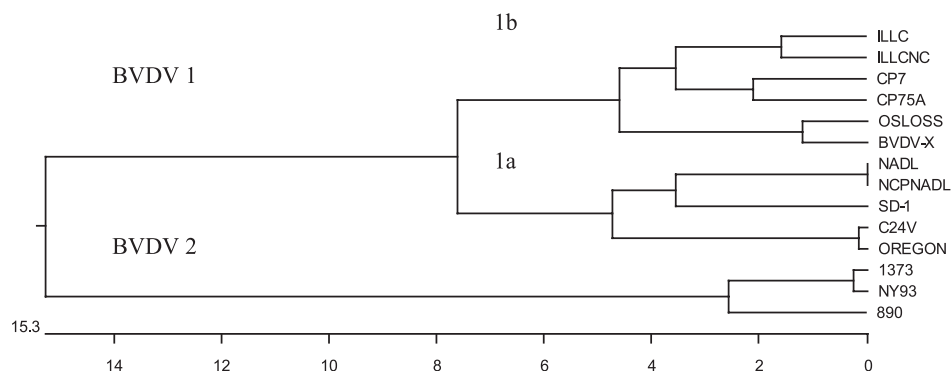To determine whether the genome of BVDV-X contains Oregon C24V-derived

Figure 1. Phylogenetic tree derived from the 5'UTR of the so-far completely sequenced BVDV 1 and BVDV 2 strains and the BVDV-X vaccine strain. The units at the bottom of the tree indicate the number of substitution events.

Table IV. Amino acid identity between proteins of the BVDV-X vaccine strain and other BVDV 1 and BVDV 2 strains.

Virus strain	Amino acid identity (%)											
	N^{pro}	C	E^{rns}	E1	E2	P7	NS2	NS3	NS4A	NS4B	NS5A	NS5B
BVDV 1 Osloss	96	97	96	94	91	95	92	98	98	94	95	97
BVDV 1 CP7	92	92	94	93	86	91	89	98	98	94	92	96
BVDV 1 ILLC	89	89	95	93	84	91	84	94	96	87	90	84
BVDV 1 NADL	86	89	89	85	77	77	78	97	92	92	93	91
BVDV 1 Oregon	86	87	91	88	76	83	77	97	93	91	84	92
BVDV 2 890	72	81	78	84	62	68	59	91	85	83	69	79

sequences, the complete nucleotide sequence of the BVDV-X genome was compared to that of BVDV Oregon C24V, in 200-nucleotide sections. The results showed that there is no region where the nucleotide similarity between the two viruses reaches 100% (Fig. 2). The highest nucleotide similarity, of 89%, was found in the NS3 region, whereas the lowest similarities, of 62.5% and 63.5%, were found in the N-terminal part of E2 and in the variable 3'V region of the 3'UTR, respectively. The average nucleotide similarity was found to be of 77.4% between BVDV-X and BVDV Oregon, whereas it was significantly higher with 93.7%, between BVDV-X and BVDV strain Osloss.

4. DISCUSSION

To control the various forms of diseases caused by BVDV, both modified live and inactivated vaccines have been commonly used since the early 1960s [10, 27]. The first vaccines were aimed at reducing the severity of clinical symptoms of postnatal BVD. After elucidation of the pathogenesis of persistent infection and MD in the mid 1980s [6, 8], and the role of PI cattle in the disease, the main goal of modern BVDV vaccines is to prevent foetal infections.

Modified live vaccines contain cp BVDV strains, and generally give better immunological response than inactivated vaccines.
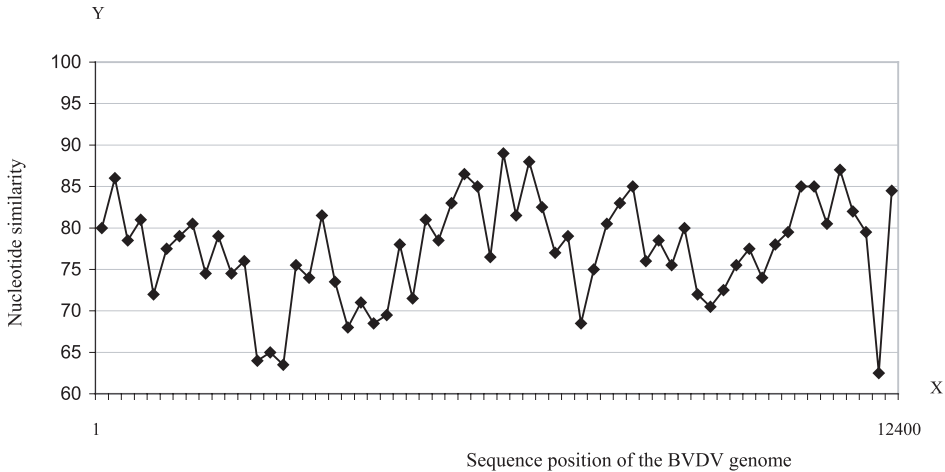
Figure 2. Graphical plot showing the nucleotide sequence similarity between BVDV-X and BVDV Oregon C24V. Axis Y shows the sequence similarity in percentage. Axis X shows the sequence positions. One section corresponds to 200 nucleotides.

Live vaccines can clinically protect immunocompetent animals against viral challenge [13]; however, they do not confer complete protection against congenital infection. Furthermore, vaccination of PI cattle may even trigger the development of MD after superinfection with a cp strain [7]. A further disadvantage is that the live vaccines contain limited antigen mass and need the possibility to replicate in the host in order to develop the necessary immunity. During replication, the live vaccine strains might recombine in the PI animal with the resident ncp strains of BVDV and lead to early [1, 5, 17] or delayed onset of postvaccinal MD, as observed in several cases [3, 16, 35]. Such a situation may cause considerable problems during control or eradication programs. The cp BVDV strains of the live vaccines can cross the placental barrier independently of the time of gestation and infect foetuses [28], but in contrast to the ncp BVDV strains, they do not induce persistent infections [9]. A further disadvantage of the live virus vaccines is their immunosuppressive effect [36].

The presence of foreign BVDV strains in permanent cell lines or in foetal calf serum batches may cause serious problems during the development and/or production of the live vaccines. If such cells or serum are used in vaccine production, the vaccine can be contaminated with ncp BVDV and become a source of spread of BVDV infections [26]. A further possible scenario is the accidental pickup of a cp strain and the continued production of the vaccine from this foreign cp virus.

To examine the questions raised in the introduction, the complete genomes of the BVDV-Xpre and one of the marketed batches (BVDV-X) have been sequenced. Data of the full-length sequence of BVDV-Xpre proved that the original strain used for the development of BVDV-X was Oregon C24V. Since correct documentation of this batch is not available, it is not known, how many in vitro and in vivo passages it has undergone, thus the role of the detected sequence differences – mainly in the E1 and E2 regions – compared to the Oregon C24V strain can not be determined.

The sequence data of the genomic sequence of BVDV-X revealed that this

virus is a BVDV 1b variant, that showed the highest average sequence homology, 93.7%, to reference BVDV strain Osloss. In contrast, the average sequence homology with BVDV Oregon C24V was sigificantly lower, 77.4%, and the detailed genome scanning revealed that the genome of BVDV-X did not contain Oregon C24V sequence. This indicates that BVDV-X did not derive from Oregon C24V, but it might have originated from a pickup of an exogenous cp BVDV strain from the in vitro or in vivo steps of passaging. It is possible that an animal used for the in vivo passaging was infected with exogenous cp BVDV, which was later re-isolated and used for vaccine production. A miss-labelling of tubes is another possibility to be taken into account. It has to be emphasized that in the historic years when BVDV-X was used as a vaccine, unequivocal methods for strain identification were not still developed, neither panels of monoclonal antibodies, nor molecular tools, like nucleotide or aa sequencing were available. Thus, the means to detect a virus-switch or a virus pickup were limited at that time. The comparative genome analysis in the present study revealed the surprising fact that BVDV-X contained a virus very different from BVDV-Xpre. Considering the major differences in the genomes of the two viruses, it is likely that neither viral recombination, nor virus-alteration, but a pickup of a foreign virus occurred in this case. It is likely that similarly to Oregon C24V, the uptaken foreign virus was also harmless and immunogenic. This could be the reason that despite the virus-switch, the BVDV-X vaccine continuously maintained its innocuity and efficacy, as proven by regular quality testings, and the presence of the foreign virus remained unnoticed over many years.

The unchanged safety and efficacy of the vaccine can be considered a lucky scenario, since the picking up of a new virus during passaging could have led to very serious consequences. Contamination of products, like vaccines or FCS, is a severe risk in the biological industry. A previous study from

our group revealed for example BVDV 2 contamination in a FCS batch used in New Zealand [40]. Since BVDV 2 has not been observed in this country before, it was important to learn that the batch of the serum originated from the USA. This example shows that biological contaminations may have a transboundary character, as a risk factor to animal and human health worldwide.

Besides the above listed dangers, it is interesting to note that in the present case the strain-switch may probably have had some "positive" practical effects. In a recent study we found that the Osloss-like virus of the BVDV-X vaccine was closely related to the cp BVDV strains isolated in the region of vaccination during the early 1970s [1]. The closer relation emphasizes that the BVDV-X immunisation presumably gave a stronger protection against the local Central European field isolates than the American Oregon C24V variant would have provided.

In summary, the molecular approach was applied in the present retrospective study to reveal an interesting intermezzo in the history of veterinary vaccinology. The nucleotide sequence analysis proved that millions of animals were immunised over two decades with a "foreign" virus strain, which was presumably picked up during the series of passages for vaccine production. Fortunately, the new virus was of low pathogenicity, as shown by the regular vaccine quality control tests. The results of this study emphasize the necessity of more precise quality testing of commercially available live virus vaccines, including monoclonal antibody analysis and sequencing, to verify their authenticity.

A further outcome of this work is that the full-length nucleotide sequence of a BVDV strain, BVDV-X has been determined, and this information will hopefully provide additional data to the panel of pestivirus biology and vaccinology.

The nucleotide sequence of the whole genome of the BVDV-X vaccine strain has

been deposited in GenBank under accession number AJ585412.

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