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Bovine herpesvirus-1: Evaluation of genetic diversity of subtypes derived from field strains of varied clinical syndromes and their relationship to vaccine strains

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ABSTRACT

Bovine herpesvirus-1 (BoHV-1) causes significant disease in cattle. Control programs in North America incorporate vaccination with modified live viral (MLV) or killed (KV) vaccine. BoHV-1 strains are isolated from diseased animals or fetuses after vaccination. There are markers for differentiating MLV from field strains using whole-genome sequencing and analysis identifying single nucleotide polymorphisms (SNPs). Using multiple primer sets and sequencing of products permits association of BoHV-1 isolates with vaccines. To determine association between vaccine virus and strains isolated from clinical cases following vaccination, we analyzed 12 BoHV-1 isolates from animals with various clinical syndromes; 9 corresponded to BoHV-1.1 respiratory group. The remaining three corresponded to BoHV-1.2b, typically found in genital tracts of cattle. Four BoHV-1 isolates were identical to a vaccine strain; three were from post-vaccination abortion episodes with typical herpetic lesions whose dams had received MLV vaccine during pregnancy, and one from a heifer given a related MLV vaccine; Sequences of two respiratory isolates perfectly matched mutations characterizing RLB106 strain, a temperature sensitive mutant used in intranasal and parenteral vaccines. The last three respiratory strains clearly appeared related a group of MLV vaccines. Previously the MLV vaccines were grouped into four groups based on SNPs patterns. In contrast with above-mentioned isolates that closely matched SNP patterns of their respective MLV vaccine virus, these 3 strains both lacked some and possessed a number of additional mutations compared to a group of MLV vaccine viral genome. Finding BoHV-1.2b in respiratory cases indicates focus should be given BoHV-1.2b as an emerging virus or a virus not recognized nor fully characterized in BRD.

1. Introduction

Bovine herpesvirus-1 (BoHV-1) is an alphaherpesvirus subfamily member that causes various disease syndromes in cattle including bovine respiratory disease (BRD), abortions, reproductive tract disease (genital tract) in the female (vulvovaginitis) and male (balanoposthitis), conjunctivitis, and severe neonatal disease [1,2]. There are three subtypes of BoHV-1: BoHV-1.1, BoHV1.2a, and BoHV1.2b [3–6] which have been differentiated by genomic DNA restriction endonuclease fragment polymorphisms [5,6]. A map has been established that identifies the essential and non-essential genes of the BoHV-1 genome [4].

Control of BoHV-1 associated disease in North America includes the use of both modified live virus (MLV) and inactivated/killed vaccines [7]. Vaccines are used primarily for the control of bovine respiratory disease and fetal disease (abortions) caused by BoHV-1 [1]. While some MLV BoHV-1 vaccines have USDA licensure for use in pregnant cows, there are restrictive conditions which may vary between companies. These conditions often require vaccination with the same product prior to breeding at a specified time before vaccination during pregnancy [7]. It has been reported recently that BoHV-1 MLV are involved in fetal loss (abortions) after pregnant cows were vaccinated during pregnancy according to instructions on the label [8–10]. BoHV-1 may be isolated from feedlot calves

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Table 1
Description of vaccine virus strains used in this study*.

Vaccine no.	Vaccine	Group based on SNPs pattern	Vaccine identification
Vax 1	Arsenal® IBR vaccine MLV	1	Ser no. 165-004
Vax 2	Titanium™ IBR vaccine MLV	1	Ser no. 3876B
Vax 3	Express® 1 IBR vaccine MLV	2	Ser no. 125-017
Vax 4	Pyramid ® IBR vaccine MLV	2	Ser no. 206116A
Vax 5	Vista® IBR vaccine MLV	2	Ser no. 90476906
Vax 6	BoviShield ®IBR vaccine MLV	3	Ser no. A249968B
Vax 7	BoviShield Gold FP® 5 MLV	3	Ser no. A827323B
Vax 8	TSV-2® Nasal MLV**	4	Ser no. A600691

* The table reproduced in modified form from *Vaccine* 31; 1471-1479, 2013.
** RLB 106 strain in TSV-2® Nasal MLV, is also in CattleMaster ®4, CattleMaster® Gold FP®, Inforce ™3.

with BRD shortly after vaccination at processing where they receive MLV vaccine and/or after receiving MLV intranasal vaccine [11,12]. Identifying whether an isolate is a vaccine strain or a field strain is relevant to clinicians and diagnosticians in the interpretation of diagnostic test results.

A recent study involving whole genome sequencing provided DNA sequence data that helps differentiate field strains from vaccine strains of BoHV-1, [13] The study showed that while there were BoHV-1 strains isolated from diagnostic samples that clearly did not match any MLV vaccine strains, there were isolates from aborted fetuses and calves with respiratory disease that shared single nucleotide polymorphisms (SNPs) pattern of particular BoHV-1 MLV vaccine strains. Based on the SNPs pattern, the MLV vaccines could be characterized into groups [13]. The are four groups listed (Table 1) [13].

The purpose of this study was to expand the investigation of BoHV-1 genetic variability by analyzing isolates from different syndromes collected from different regions of the U.S.

2. Materials and methods

2.1. BoHV-1 virus vaccines and clinical isolates

The grouping and genomic characteristics of the BoHV-1 vaccine strains referred to in the present study have been described [13]. In that study there were four vaccine groups based on SNPs patterns and are listed in Table 1 [13]. During the study there were viruses with their genomes aligned to the BoHV1.1 Cooper reference genome with only a 97.5% nucleotide identify detected with these genomes and BoHV1.1. These genomes were then aligned with the BoHV1.2b genome. Furthermore, several previously typed clinical isolates found to be unrelated to any vaccine strain [13] are included in this study for comparison purposes (Table 2, cases C4, C5 & C11–14). An additional 12 isolates were examined in the present study. A brief clinical history associated with each are provided in Table 3 as cases C15–26.

Nasal swabs were tested by virus isolation on MDBK cells as described using BVDV-free bovine fetal serum [13] and in selected cases positive samples were titrated to establish relative concentration. Isolates C15 and C16 were isolated at the Willard Sparks Food Animal Research Center, Oklahoma State University from calves treated for BRD. The viral concentration in the nasal swabs of these two calves was $10^{6.3}$ and $10^{4.8}$ $TCID_{50}$, respectively, using the viral titration as described [14]. The calves had been given a Group 2 vaccine at entry 12 days prior. Strain C17 as isolated from a heifer's whole blood after vaccination with a Group 3 vaccine. The C18, C19, and C20 strains were isolated at necropsy from California cattle and supplied by the University of California Davis CAHFS Veterinary Diagnostic Laboratory, Tulare, CA; vaccination histories for each were not available. Strain C18 was from a 4 year-old dairy cow with BRD lesions observed at necropsy; C19 was from a 12 day-old dairy calf with BoHV-1 viremia and lesions in the lung, liver, and adrenal; and C20 was from a 12 day-old dairy calf with BoHV-1 bronchopneumonia. Strain C21 was from a New York BRD study with dairy calves and was provided Cornell University, Ithaca, NY. This nasal isolate was from a calf with BRD signs and had been given an intranasal MLV vaccine (containing BoHV-1, parainfluenza-3 virus, and bovine respiratory syncytial virus) the same day as the nasal swab was collected. Another calf from the New York study was positive for BoHV-1 but the viral DNA did not separate from the cellular DNA during the ultracentrifugation process and so was not sequenced. Strain C22 was isolated from a beef calf in an Oklahoma feedlot, the nasal swab being collected when the calf was initially treated. The calf had received a Group 2 vaccine at feedlot entry. The calf had been purchased at an auction market and any prior vaccination history was unknown. Strain C23 was supplied by the Colorado State University Veterinary Diagnostic Laboratory, Fort Collins, CO. This isolate was from an aborted fetus with confirmed histological lesions typical of BoHV-1. Its dam had received a Group 3 MLV vaccine during pregnancy. Isolates C24 and C25 strains were from the University of Wyoming State Veterinary Laboratory, Laramie, WY and the isolates were from aborted fetuses in two different herd outbreaks of post-vaccination abortion outbreaks. Both fetuses had confirmed histological lesions confirmed typical of BoHV-1 infections. The dams in both instances had been given an MLV vaccine from Group 3. Strain C26 was supplied by the Texas A and M Veterinary Medical Diagnostic Laboratory, Amarillo, TX, and was from a feedlot calf with severe, fatal BoHV-1 respiratory disease. The calf had been given a Group 3 MLV at feedlot entry.

Table 2
Description of reference strain and BoHV-1 clinical isolates*.

Reference/Case	Virus identification	History
Ref 1	IBR Cooper, USDA NVSL challenge 97-11 (GenBank accession JX898220)	USDA Challenge strain
Case 4 (C)	BI 3025-2502	Larynx and trachea isolate. Respiratory disease and vulvovaginitis, tracheitis and bronchopneumonia
Case 5	CSU 034-26826	Aborted fetus—fetal tissues; dam vaccinated during pregnancy with MLV vaccine
Case 11	CSU 5350-292930	Lung and trachea, respiratory disease, feedlot
Case 12	CSU 5333-291972	Lung and trachea, respiratory disease, feedlot
Case 13	CSU 31751	Respiratory disease, feedlot
Case 14	CSU 034-10640	Vaginal mucosa, necrotic vaginitis, dairy cow

* Selected information in the table from *Vaccine* 31; 1471–1479, 2013.