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Whole-genome sequence analysis reveals unique SNP profiles to distinguish vaccine and wild-type strains of bovine herpesvirus-1 (BoHV-1)

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ABSTRACT

Bovine herpesvirus-1 (BoHV-1) is a major pathogen affecting cattle worldwide causing primarily respiratory illness referred to as infectious bovine rhinotracheitis (IBR), along with reproductive disorders including abortion and infertility in cattle. While modified live vaccines (MLVs) effectively induce immune response against BoHV-1, they are implicated in disease outbreaks in cattle. Current diagnostic methods cannot distinguish between MLVs and field strains of BoHV-1. We performed whole genome sequencing of 18 BoHV-1 isolates from Pennsylvania and Minnesota along with five BoHV-1 vaccine strains using the Illumina Miseq platform. Based on nucleotide polymorphisms (SNPs) the sequences were clustered into three groups with two different vaccine groups and one distinct cluster of field isolates. Using this information, we developed a novel SNP-based PCR assay that can allow differentiation of vaccine and clinical strains and help accurately determine the incidence of BoHV-1 and the association of MLVs with clinical disease in cattle.

1. Introduction

Bovine herpesvirus-1 (BoHV-1) causes significant health concerns in cattle including respiratory disease, fetal anomalies, and reproductive tract infections worldwide (Fulton et al., 2013). BoHV-1 has been known to cause disease in cattle for many years, and the first report of the disease believed to be caused by BoHV-1 came from Germany in the 19th century (Graham, 2013). BoHV-1 is one of the major etiological agents of Bovine Respiratory Disease (BRD) complex or "shipping fever" which is one of the most economically important disease of cattle in North America (Jones and Chowdhury, 2007). A 2011 study estimated that 21.2% of beef cattle (2.29 million) in feedlots were affected by BRD, which was responsible for approximately 45–55% of all deaths in the feedlot (Johnson and Pendell, 2017). In addition to the economic losses, BoHV-1-free status is an important aspect in the international trade as all semen used in artificial insemination must be sourced from BoHV-1 seronegative bulls (O'Grady et al., 2008). Multiple factors including virus virulence, age of the host and concurrent bacterial infections determine the outcome of BoHV-1 infection and the severity of clinical disease (Muylkens et al., 2007). A notable clinical sign of BoHV-1 infection in dairy cows is a significant milk drop followed by respiratory and ocular signs characterized by congestion of nasal mucosa, serous to mucopurulent nasal discharge, increased respiration and cough in severe cases (Graham, 2013).

A key challenge in the control of BoHV-1 infection is latent infection with this virus in cattle. After acute infection BoHV-1, like other *alphaherpesviruses,* establishes latent infection in cattle primarily in sensory neurons within trigeminal ganglia (TG) or dorsal root ganglia (Jones, 1998, 2003) and the animals remain carriers for the rest of their life. When cattle with latent BoHV-1 infection are exposed to natural stressors like travel, extreme weather conditions, overcrowding, or immunosuppressive treatments such as dexamethasone (DEX), reactivation of latent virus occurs, which leads to virus shedding and spread to susceptible hosts (Preston and Nicholl, 2008; van Oirschot, 1995; Winkler et al., 2000, 2002).

BoHV-1 is a large, enveloped double stranded DNA virus belonging to the sub family *Alphaherpesvirinae* within the family *Herpesviridae* (Chase et al., 2017; Jones and Chowdhury, 2007; Levings and Roth, 2013). BoHV-1 virions are approximately 200–300 nm in diameter, which consist of a central core with a single linear dsDNA molecule

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surrounded by an icosahedral capsid of about 125 nm diameter containing 162 capsomeres (150 hexons and 12 pentons) (MacLachlan et al., 2017).

Three genetic subgroups of BoHV-1 have been defined. Viruses belonging to BoHV-1.1 subgroup are associated with respiratory and reproductive disease in Europe, North America and South America. Members of BoHV-1.2a subgroup are prevalent in Brazil and are associated with respiratory and reproductive disease and in particular, localized genital infections [infectious pustular vulvovaginitis (IPV) in females and infectious balanoposthitis (IPB) in males]. Subgroup BoHV-1.2b viruses are less virulent than BoHV-1.1 viruses and are found in Europe, USA and Australia. BoHV-1.2b viruses are associated with respiratory disease and localized IPV and IPB genital infections (Chase et al., 2017; d'Offay et al., 2016; Jones and Chowdhury, 2007).

In general BoHV-1 vaccination using MLVs and inactivated viral vaccines have been found to effective (Chase et al., 2017). However, several evidences pointed out that vaccination with BoHV-1 MLVs could result in disease outbreaks including abortions in vaccinated pregnant cattle. For example, it was found that MLV BoHV-1 vaccines caused abortions in BoHV-1 naïve and vaccinated pregnant cattle just like the field virus (Chase et al., 2017; O'Toole et al., 2014, 2012). Further, BoHV-1 has been isolated from feedlot calves with BRD shortly after they received BoHV-1 MLV vaccines (Fulton, 2009). Therefore, it is highly important to identify whether a BoHV-1 isolate is a vaccine strain or a wild-type field strain for better interpretation of diagnostic test results (Fulton et al., 2016). Novel diagnostic methods to distinguish the field and vaccine BoHV-1 strains would allow accurate determination of the prevalence of BoHV-1 infection in cattle. In addition, such methods would also help investigate the association of MLV with clinical disease and to evaluate the need for improved BoHV-1 vaccines.

Next-generation sequencing (NGS) methods provide low cost and reliable large-scale DNA sequencing (Metzker, 2010) and provide greater sequencing coverage for accurate genotyping. Recent studies showed that comparing the whole genome sequences of BoHV-1 isolates helps differentiate BoHV-1 wild-type field strains from vaccine strains; single nucleotide polymorphism (SNP) profiles were used to group BoHV-1 isolates into distinct clusters (Fulton et al., 2013, 2015).

The currently used molecular diagnostic methods cannot distinguish between vaccine and field BoHV-1 strains from a clinical case. Hence, there is an urgent need to develop molecular diagnostic methods that would allow not only accurate BoHV-1 diagnosis but also help identify if the given clinical case is caused by a wild-type or vaccine strain of BoHV-1. Here we report the development of a SNP-based PCR assay that would allow differentiation between vaccine and clinical strains of BoHV-1.

2. Materials and methods

2.1. BoHV-1 field isolates and vaccines

A total of five most widely used commercial BoHV-1 vaccine strains and 18 field isolates were sequenced in this study (Tables 1 and 2). Clinical samples submitted to the veterinary diagnostic laboratories at the University of Minnesota and Pennsylvania State University, as nasal, vaginal, eye, or bronchus swabs (Sigma Virocult™ swabs-Medical Wire and Equipment, England) or as tissue homogenates or stomach contents were used for virus isolation. All the five vaccines were obtained from commercial suppliers.

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

Sample ID	Manufacturer	Vaccine
V1	Merck	Vista® 3 SQ
V2	Boehringer Ingelheim	Pyramid® IBR
V3	Zoetis	Bovi-Shield GOLD® IBR-BVD
V4	Boehringer Ingelheim	Express™ I
V5	Pfizer	TSV-2™

Table 2
Description of clinical isolates used in this study.

Case ID	Date of Diagnosis	Type of Sample	GenBank Accession Number
S1	3/5/2015	Nasal swab	MG407791
S2	3/5/2015	Nasal swab	MG407792
P1	3/25/2015	Nasal swab	MG407790
D1	5/15/2001	Tissue Homogenate	MG407775
D2	7/17/2001	Tissue Homogenate	MG407776
D3	9/5/2001	Tissue Homogenate	MG407777
D5	5/13/2008	Stomach Contents	MG407778
D6	4/10/2009	Tissue Homogenate	MG407779
D7	10/4/2010	Tissue Homogenate	MG407780
D8	10/4/2010	Tissue Homogenate	MG407781
D9	10/4/2010	Tissue Homogenate	MG407782
D10	10/26/2010	Eye Swab	MG407783
D11	10/26/2010	Eye Swab	MG407784
D12	11/23/2010	Nasal Swab	MG407785
D13	11/23/2010	Nasal Swab	MG407786
D15	6/9/2011	Nasal Swab	MG407787
D17	5/2/2012	Nasal Swab	MG407788
D19	11/19/2013	Nasal Swab	MG407789

2.2. Virus isolation and viral DNA extraction

The field isolates were subjected to viral DNA extraction using QIAamp MinElute Virus Spin Kit (Qiagen, USA). The total viral genomic DNA was used in SYBR® Green based Real Time Polymerase Chain Reaction (qPCR) assay to detect presence of BoHV-1. Previously defined primers were used to amplify a portion of the glycoprotein H gene of BoHV-1: forward: 5′-GTA AGG GTA TAT TGA TTG C-3′ and reverse:5′-GAC AGT GAG TAT GAG GAC-3′ (GenBank accession number X58867) (Herlekar et al., 2013). The BoHV-1 qPCR positive isolates were subjected to virus isolation on Madin-Darby Bovine Kidney (MDBK) cells maintained in Dulbecco's Modified Eagle's medium (DMEM) with 2% fetal bovine serum and 1% Antibiotic-Antimycotic (100×). The infected cells were put through three freeze-thaw cycles, and the resulting supernatant was subjected to ultracentrifugation to obtain concentrated virus as previously described (Ichim and Wells, 2011). Briefly, OptiSeal bell-top ultracentrifuge tubes (Beckman Coulter, USA) were sterilized by exposure to ultraviolet radiation in a biological safety cabinet for 20 min. 30 mL of viral supernatant was centrifuged at 16,500 rpm for 90 min at 4 °C in a Beckman Coulter ultracentrifuge (Type 50.2 Ti Rotor). Following centrifugation, the medium was slowly decanted. The virus pellet was re-suspended in 1000 µl of DMEM media. 200 µl of the virus supernatant was subjected to viral DNA extraction using the QIAamp MinElute Virus Spin Kit (Qiagen, USA).

2.3. BoHV-1 whole genome sequencing

Whole genome sequencing was performed at the Penn State Genomics Core Facility, University Park, Pennsylvania. The BoHV-1 DNA barcoded libraries were made using the Illumina TruSeq DNA Nano kit. An equimolar pool of the libraries was sequenced on a single 150 × 150 paired-end run on an Illumina MiSeq. Approximately 700,000 pairs of reads or 210 MB of sequence was generated per isolate.

2.4. Data analysis

Sequence quality was visualized using FastQC reports. All the field isolates were mapped to National Veterinary Services Laboratory (NVSL) reference/challenge Cooper strain of BoHV-1.1. (JX898220) using bwa-mem algorithm. Mapped data were then used for variant calling. Variants were called using freebayes (v1.0.2) program. Low