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Genomic and antigenic characterization of bovine parainfluenza-3 viruses in the United States including modified live virus vaccine (MLV) strains and field strains from cattle

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ABSTRACT

This study investigated the genetic and antigenic characterization of parainfluenza-3 virus (PI3V) of cattle. Using molecular tests including real time PCR and viral genome sequencing, PI3V strains could be separated into PI3V types, including PI3V A, PI3V B, and PI3V C. Isolates from cattle with bovine respiratory disease clinical signs and commercial vaccines in the U.S. with MLV PI3V were typed using these molecular tests. All the MLV vaccine strains tested were PI3V A. In most cases PI3V field strains from calves receiving MLV vaccines were types heterologous to the vaccine type A. Also antigenic differences were noted as PI3V C strains had lower antibody levels than PI3V A in serums from cattle receiving MLV PI3V A vaccines. This study further demonstrates there is genetic variability of U.S. PI3V strains and also antigenic variability. In addition, isolates from cattle with BRD signs and receiving MLV vaccines may have heterologous types to the vaccines, and molecular tests should be performed to differentiate field from vaccine strains. Potentially the efficacy of current PI3V A vaccines should be evaluated with other types such a PI3V B and PI3V C.

1. Introduction

Bovine respiratory diseases (BRD) have etiologies of viruses, bacteria, and *Mycoplasma* spp. (Fulton,2008, 2009) Viruses included in BRD, either singly or in combination include bovine herpesvirus-1 (BoHV-1), parainfluenza −3 virus (PI3V), bovine viral diarrhea viruses (BVDV), bovine respiratory syncytial virus (BRSV), and bovine coronaviruses (BoCV)(Fulton,2008, 2009; Fulton et al., 2011; Fulton et al., 2013; Fulton et al., 2016). The bovine PI3V is a RNA virus in the genus *Respirovirus* in the family Paramyxoviridae (Chanock et al., 2001). In North America both killed/inactivated and modified live virus (MLV) vaccines are used for control of these viruses (Compendium of Veterinary Products, 2010). Clinicians seeking information on potential causative agents/etiologies often submit samples from cattle such as nasal swabs or blood samples from affected cattle and tissues from necropsy samples to the diagnostic laboratories. Samples may be tested by “traditional tests” including virus isolation for infectious virus or more often “modern tests”, molecular tests including polymerase chain reaction (PCR) and in some cases, sequencing of the agent’s genome (Fulton et al., 2016; Fulton and Confer, 2012). Interpretation of such results is important for clinicians and the diagnostic laboratory. Viruses may appear to be similar based on the test used such as infectious viruses from cultures or these from the molecular tests that are homologous based on the product produced using primers. Also there is the dilemma of whether the virus identified is a field virus or a vaccine strain. A recent study utilized samples from cattle with BRD signs after processing with MLV vaccines administered (Fulton et al., 2016). In that study there were isolates of BoHV-1, BVDV, and PI3V that utilized sequencing of the viral genome and found either field strains or vaccine strains. Thus it is important to be able to differentiate viral isolates of field or vaccine origin. Several PI3V strains in the U.S. have been examined using viral sequencing with three types identified, PI3VA, PI3VB, and PI3V C. (Neill et al., 2015)

The study purposes were three-fold: (1) characterize PI3V strains in available commercial vaccines by molecular tests, (2) compare PI3V strains from field cases of BRD by molecular tests and to determine if the strain was related to MLV strains, and (3) using serums from calves given MLV vaccines containing PI3VA strains to determine antigenic variation when serums were tested in viral neutralization test with PI3VA and two PI3V C strains.

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Table 1
PI3V Isolates from BRD clinical cases.

Study	Year	Identifcation	Collection site	Days after processing	MLV vaccine at procesing	Type by PCR
Study 1	1999	OK P 100	NS[a]	28	No PI3V MLV	PI3VA
		OK P 141	NS	28	No PI3V MLV	PI3VB
		OK P 147	NS	28	No PI3V MLV	PI3VA
		OK P 242	NS	33	No PI3V MLV	PI3VA
Study 2	2009	OK 261 SP	NS	11	Vax 1	PI3VC
		OK 284 SP RLU	Right lung	30	Vax 1	PI3VC
		OK 309 SP LLU	Left lung	18	Vax 1	PI3VC
		OK 453 SP RLU	Right lung	24	Vax 1	PI3VC
		OK 467 SP RLU	Right lung	17	Vax 1	PI3VC
		OK 643 SP LLU	Left lung	35	Vax 1	PI3VC
		OK 643 SP RLU	Right lung	35	Vax 1	PI3VC
Study 3	2012	OK PR 2 NS	NS	2	Vax 2	PI3VB
		OK PR 7 NS	NS	2	Vax 2	PI3VC
Study 4	2014	OK P 5797	NS	12	Vax 3	PI3VC
		OK P 778	NS	20	Vax 4	PI3VC
		OK P 825	NS	20	Vax 3	PI3VC
		OK P 438	NS	19	Vax 3	PI3VC
		OK P 831	NS	20	Vax 3	PI3VC
		OK P 9054	NS	NA	Vax 3	PI3VC
		OK P NM 64	NS	18	Vax 5	PI3VA
		OK 95806	NS	13	Vax 6	PI3VB
		OK 95524	NS	15	Vax 6	PI3VB

[a] NS-nasal swab.

2. Materials and methods

2.1. Cattle groups

Viruses from cattle in this study included 22 viruses from 4 different studies (Table 1) dated from 1999 until 2014. Study 1 were cattle from the Southeast (SE) region of the U.S. shipped to an experimental feed yard in New Mexico. (Fulton et al., 2001) These cattle had not received PI3V vaccination prior to shipment. Study 2 included cattle from the SE U.S shipped to the Willard Sparks Experimental Feedyard at Oklahoma State University in 2009. Samples included both nasal swabs from cattle with BRD signs and samples collected from lungs at necropsy. These cattle had received a MLV vaccine containing PI3V at entry to the feed yard. Study 3 samples were from an Oklahoma feed yard from cattle with BRD signs and they had received a MLV vaccine at entry to the feed yard. The Study 4 included isolates from cattle using nasal swabs from cattle with BRD signs (Fulton et al., 2016). For the Studies 2–4 the vaccine used in each particular study is listed in Table 1.

2.2. Viruses from field studies

The isolates from Table 1 from Studies 1 and 4 were grown in cell culture using MDBK or bovine turbinate monolayers and confirmed by direct fluorescent antibody tests and/or a gel based PCR test. (Fulton et al., 2016; Fulton et al., 2001) Isolates from Studies 2 and 3 were grown in MDBK monolayers and confirmed as PI3V using gel based PCR test (Fulton et al., 2016)

2.3. Vaccines used in PCR and genomic characterization

There were 7 different MLV vaccines available in the U.S. used for molecular testing and which are listed in Table 2. (Compendium of Veterinary Products, 2010) These included Vax 1, 2, 4,5,7,8, and 9. Vax 3 and Vax 6 represented vaccines among several different ones given to calves in Study 4, and did not contain PI3V.

Table 2
MLV vaccines with PI3VA.

Vaccine no.	Vaccine
Vax 1	BRD Shield ™
Vax 2	Pyramid ® 5 MLV
Vax 3	Pyramid ® 3 MLV
Vax 4	Titanium ™ 5 MLV
Vax 5	BoviShield Gold 5 ® MLV
Vax 6	BoviShield IBR-BVD ® MLV
Vax 7	Vista ® 5 SQ MLV
Vax 8	Express ® 5 MLV
Vax 9	Arsenal ® 4.1 MLV

2.4. Molecular tests

2.4.1. Genomic sequencing of PI3V strains

Sequence analysis of BPI3V genomic RNAs was done as previously described (Neill et al., 2014) and as modified for use on the Illumina MiSeq (Hause et al., 2015). Sequences were assembled using the LaserGene NextGen suite (DNASTAR, Madison, WI). Comparison of sequences and generation of phylogenetic trees were done using MEGA 7.0 (Kumar et al., 2016) .The viral sequence was a region of the viral nucleocapsid and had 1548 bases.

2.4.2. PI3V real time PCR

PI3V isolates were genotyped by differential real-time PCR. Three primer/probe sets were used. The PI3V sets are illustrated in Table 3. Because of the amount of genetic diversity in PI3V A viruses, a PI3V universal set was designed that detected all isolates. PCR primer and probe sets for type B and C were designed that were specific for the respective genotypes (Table 3). The real time PCR primers were designed within the phosphoprotein (P) gene. PCR reactions were assembled using 0.4 µM of each primer and 0.2 µM probe in a 25 µl reaction using the QuantiFast Probe RT-PCR Kit (Qiagen, Inc., Valencia, CA). One microliter of cell culture fluid containing the virus was used as template. Cycling conditions were: 50 °C for 20 min, 95 °C for 5 min, then 40 cycles of 95 °C for 15 s and 62 °C for 30 s. Fluorescence was read following every cycle. The isolate was considered positive for a specific genotype if the Ct values were between 15 and 35. Above 35,