Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Vaccine 34 (2016) 3478–3492

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Detection and characterization of viruses as field and vaccine strains in feedlot cattle with bovine respiratory disease

R.W. Fulton[a,*], J.M. d'Offay[a], C. Landis[a], D.G. Miles[b], R.A. Smith[c], J.T. Saliki[d], J.F. Ridpath[e], A.W. Confer[a], J.D. Neill[e], R. Eberle[a], T.J. Clement[f], C.C.L. Chase[f], L.J. Burge[a], M.E. Payton[g]

[a] Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK 74078, USA
[b] Veterinary Research and Consulting Services, Greeley, CO 80634, USA
[c] Veterinary Research and Consulting Services, Stillwater, OK 74075, USA
[d] Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, GA 30602, USA
[e] U.S. Department of Agriculture, Agricultural Research Service, National Animal Diseases Center, Ames, IA 50010, USA
[f] Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD 57007, USA
[g] Department of Statistics, Oklahoma State University, Stillwater, OK 74078, USA

ARTICLE INFO

Article history:
Received 16 December 2015
Received in revised form 5 April 2016
Accepted 7 April 2016
Available online 20 April 2016

Keywords:
Bovine respiratory viruses
MLV vaccines
Diagnostic virology

ABSTRACT

This study investigated viruses in bovine respiratory disease (BRD) cases in feedlots, including bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine coronaviruses (BoCV) and parainfluenza-3 virus (PI3V). Nasal swabs were collected from 114 cattle on initial BRD treatment. Processing included modified live virus (MLV) vaccination. Seven BRD necropsy cases were included for 121 total cases. Mean number of days on feed before first sample was 14.9 days. Swabs and tissue homogenates were tested by gel based PCR (G-PCR), quantitative-PCR (qPCR) and quantitative real time reverse transcriptase PCR (qRT-PCR) and viral culture. There were 87/114 (76.3%) swabs positive for at least one virus by at least one test. All necropsy cases were positive for at least one virus. Of 121 cases, positives included 18/121 (14.9%) BoHV-1; 19/121 (15.7%) BVDV; 76/121 (62.8%) BoCV; 11/121 (9.1%) BRSV; and 10/121 (8.3%) PI3V. For nasal swabs, G-PCR (5 viruses) detected 44/114 (38.6%); q-PCR and qRT-PCR (4 viruses) detected 81/114 (71.6%); and virus isolation detected 40/114 (35.1%). Most were positive for only one or two tests, but not all three tests. Necropsy cases had positives: 5/7 G-PCR, 5/7 q-PCR and qRT-PCR, and all were positive by cell culture. In some cases, G-PCR and both real time PCR were negative for BoHV-1, BVDV, and PI3V in samples positive by culture. PCR did not differentiate field from vaccines strains of BoHV-1, BVDV, and PI3V. However based on sequencing and analysis, field and vaccine strains of culture positive BoHV-1, BoCV, BVDV, and PI3V, 11/18 (61.1%) of BoHV-1 isolates, 6/17 (35.3%) BVDV isolates, and 1/10 (10.0%) PI3V identified as vaccine. BRSV was only identified by PCR testing. Interpretation of laboratory tests is appropriate as molecular based tests and virus isolation cannot separate field from vaccine strains. Additional testing using sequencing appears appropriate for identifying vaccine strains.

1. Introduction

Bovine respiratory diseases (BRD) are associated with infections and complicated by stresses [1,2]. In addition to bacteria including *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma spp*. BRD etiologies include bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea viruses (BVDV), bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI3V) and bovine coronaviruses (BoCV) [1–4]. In North America killed/inactivated and modified live virus (MLV) vaccines are used for BRD control [5].

Cattle with BRD may not exhibit clinical signs or lesions definitive for specific etiology. Veterinarians use diagnostic laboratories for diagnosis using samples from affected animals. There are two primary ways diagnostic laboratories and veterinarians collaborate on laboratory testing for BRD infectious agents in feedlot cattle: testing of submitted samples from fatal cases collected at necropsy and ante mortem samples such nasal or nasopharyngeal swabs, tracheal washing materials, and peripheral blood. Such testing permits the detection of multiple agents including viruses and bacteria,

* Corresponding author. Tel.: +1 405 744 8170; fax: +1 405 744 5275.
E-mail address: Robert.fulton@okstate.edu (R.W. Fulton).

http://dx.doi.org/10.1016/j.vaccine.2016.04.020
0264-410X/

Table 1
Description of vaccines and immunogens.

Vaccine no.	Vaccine	BoHV1.1 strain	BVDV strain 1	BVDV strain 2	PI3V strain	BRSV strain
Vax 1	BoviShield Gold 5® MLV	C-13	NADL 1a CP	53637 2a CP	Not designated	375
Vax 2	BoviShield® IBR-BVD MLV	C-13	NADL 1a CP	53637 2a CP	Not in vaccine	Not in vaccine
Vax 3	Pyramid 3® MLV	Cooper	Singer 1a CP	5912 2a CP	Not in vaccine	Not in vaccine
Vax 4	Titanium 3™ MLV	Baker	C24V 1a CP	296 2a CP	Not in vaccine	Not in vaccine
Vax 5	Titanium 5™ MLV	Baker	C24V 1a CP	296 2a CP	Abbott	Lehmkul

depending on the tests available. Several studies using samples collected at necropsy and tested for several viruses by a variety of tests are listed [6–10]. Studies testing for multiple viruses from respiratory materials affected cattle ante mortem such as swabs included the following are listed [9–12]. For many years "traditional tests", culture assays were used to identify viruses, however "modern tests", molecular tests have become commonplace. Historically, the mainstay for virology workups on BRD cases was a combination of immunofluorescence and attempted viral isolation. In the past two decades, molecular assays have increasingly supplanted classical methods. Strengths and weaknesses of traditional and molecular tests and interpretation were reported [13]. Issues facing clinicians includes interpretation of positive tests from animals with BRD signs with prior MLV vaccination. Discretion should be exercised in the interpretation of laboratory tests of recently MLV vaccinated cattle with BRD. Does the identified virus or positive nucleic acid represent virulent field infections or MLV strains?

Study purposes were three fold: (1) testing nasal swabs from cattle treated for BRD and receiving MLV vaccines and necropsy tissues with varied tests including culture and molecular tests, (2) testing of culture positives determining specific type, subgenotype, or clade by viral sequencing; and (3) determining if specific viruses were field or MLV strain.

2. Materials and methods

2.1. Cattle groups

Groups included samples submitted in the fall of 2015 and represented samples collected over a period of time and then delivered for testing. Groups 1, 2, and 3 were nasal swabs collected from cattle pulled for initial BRD treatment. Samples included seven animals that died in Groups 2 and 3. Cattle received MLV vaccines at entry. Vaccines used are listed in Table 1. In Tables 2–4 , rectal temperatures at collection were recorded (information not in Group 1), day on feed (DOF) of collection and vaccination. Similar information was recorded for necropsy cases (Table 5). Samples were submitted to the Department of Veterinary Pathobiology, Oklahoma State University, and Stillwater, OK testing of viruses. Cattle received MLV vaccines containing BoHV-1, BVDV1a, and BVDV2a predominately; however limited numbers received BoHV-1, BVDV1a, BVDV2a, PI3V, and BRSV.

2.2. Virus isolation in cell culture and typing of viruses

Nasals swabs and tissues homogenates were sent to Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, GA. Virus isolation was performed as modified [14]. Inoculums were adsorbed onto one-day-old monolayers of Madin Darby Bovine Kidney (MDBK) cells in 24-well plates for 2 h at 35.5 °C. Medium containing 5% fetal bovine serum was added to monolayers and incubated at 35.5 °C. Plates were examined for cytopathic effect (CPE) for 6 days, then cells were trypsinized and tested for non-cytopathic (NCP) BVDV by direct fluorescent antibody technique (DFAT). When CPE was observed, remaining cells were trypsinized, transferred onto spot slides, and stained by DFAT using commercial fluorescein isothiocyanate-conjugated antibodies against BVDV, BoHV-1, BRSV, or bovine PI3V (Veterinary Medical Research and Development, Pullman, WA). Cultures showing CPE were frozen, clarified by centrifugation ($1000 \times g$), and supernatant saved as virus isolate. With no CPE observed, cells were subcultured by trypsinization for second 6-day passage, then tested for BVDV before reported as negative. Also, inoculums were tested at Oklahoma State University with MDBK observed for CPE and tested for BVDV by gel-based PCR (G-PCR) [6,15–17], Inoculums were tested for BoCV using human rectal tumor cell monolayers [3,4] with cultures observed 6 days. Cultures were, regardless of presence/absence of CPE tested by G-PCR [3,4,6]. Only the initial HRT monolayer passage was used in the study.

2.3. Polymerase chain reaction tests

Nasal swabs and tissue homogenates were tested for nucleic acid products by G-PCR tests for bovine viruses. Viral nucleic acids for swabs and homogenates were processed as described [18]. BoHV-1 G-PCR amplified 390-bp fragment in the UL19 gene [19,20]. PCR product for BRSV G-PCR was 481-bp F protein region and BoCV assay detected 251-bp region of N gene. [3,4,6]. BVDV primers were designed for NS5B gene and detected BVDV1 and 2 but did not distinguish subgenotypes. [6,15–17]. PI3V G-PCR primers detected 400-bp region of F gene. [21]

The quantitative real time PCR testing (q-PCR for DNA virus, BoHV1.1 and quantitative reverse transcriptase PCR [qRT-PCR] for the RNA viruses) was performed by the Animal Disease Research and Diagnostic Laboratory/Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD. Viral nucleic acid was collected as described [22]. The qRT-PCR and q-PCR detected two viruses simultaneously (BoHV-1 and BVDV, BRSV and BoCV). Ct values below 35 were considered positive while those from 35 to 40 were suspect. Negatives were those with no Ct detected or Ct values above 40. BVDV RT-PCR primers targeted a 5′-UTR [23]. BoHV-1 primers detected region of thymidine kinase (TK) [24,25]. RT-PCR BRSV detected conserved N gene region [26]. BoCV RT-PCR detected an amplified S protein region encoded by ORF 4. [27,28]

2.4. Virus sequencing

Viral sequencing for BVDV, BoCV, and PI3V was performed at the U.S. Department of Agriculture Service, National Animal Diseases Center, Ames, IA. BVDV genotyping was based on comparison of 5′-UTR [6,15–17] with reference strains, and strains in commercially available BVDV vaccines. [29] In North America CP strains in MLV BVDV vaccines include BVDV1a-NADL, BVDV1a-C24V, BVDV1a-NADL, BVDV2a-296c, BVDV2a-5912, BVDV2a-53637, BVDV2a-125A [29]. BoHV-1 culture isolates were analyzed by whole genome sequencing for single nucleotide polymorphisms and sequencing of PCR products using different PCR primers. [19,20,30]. BoHV-1 testing identifies vaccine strains and distinguishes them from field strains. PI3V testing used near complete genome sequencing

Table 2
Summary of virus isolation, PCR testing and viral sequencing from nasal swabs of BRD treated calves recently vaccinated with MLV vaccine VAX 1 – Group 1.

ID[a]	DOF[b]	Origin	BoHV-1					BVDV				
			Gel PCR	Culture	Sequence	RT-PCR CT	RT-PCR results	Gel PCR	Culture	Sequence	RT-PCR CT	RT-PCR results
2208-64	18	East Texas	Positive	Positive	MLV Vax 1	,29.32[c]	Positive	Negative	Negative		,	Not detect
2212-39	14	East Texas	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2213-3	13	East Texas	Positive	Negative		,26.37	Positive	Negative	Negative		,	Not detect
2218-70	6	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2218-54	6	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2198-377	25	Clovis, NM	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2163-251	94	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2203-442	24	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
No tag	NA[d]	No info	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2205-449	25	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2205-441	25	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2209-426	17	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2212-30	15	East Texas	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2213-14	14	East Texas	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2214-10	NA	East Texas	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2217-10	5	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2217-8	5	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,33.23	Positive
2217-31	5	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2217-32	5	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2218-93	5	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2218-9	5	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2219-34	2	Clovis, NM	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2219-11	2	Clovis, NM	Positive	Positive	MLV Vax 1	,	Not detected	Negative	Negative		,	Not detect
2143-392	99	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2206-336	26	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2209-437	20	Mississippi	Negative	Negative		,36.08	Suspect	Negative	Negative		,	Not detect
2209-455	20	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2218-51	7	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2218-74	7	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2218-20	7	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2219-17	4	Clovis, NM	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2220-487	4	Clovis, NM	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2209-436	21	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2212-24	18	East Texas	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2212-1	18	East Texas	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2212-38	18	East Texas	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2217-18	8	Mississippi	Positive	Negative		,33.43	Positive	Negative	Positive	BVDV1a NCP non MLV	,30.03	Positive
2218-6	8	Mississippi	Positive	Positive	MLV Vax 1	,23.62	Positive	Negative	Negative		,	Not detect
2218-42	8	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2218-75	8	Mississippi	Negative	Negative		,	Not detected	Positive	Positive	BVDV1b NCP	,28.94	Positive
2218-35	8	Mississippi	Negative	Negative		,36.99	Suspect	Negative	Negative		,	Not detect
2218-18	8	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2218-62	8	Mississippi	Positive	Negative		,31.64	Positive	Negative	Negative		,	Not detect
2218-16	8	Mississippi	Negative	Negative		,	Not detected	Negative	Negative		,	Not detect
2218-69	8	Mississippi	Negative	Negative		,	Not detected	Negative	Positive	BVDV1a NCP non MLV	,30.18	Positive
2219-38	5	Clovis, NM	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
2219-19	5	Clovis, NM	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
2220-481	5	Clovis, NM	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
2140-254	4	Clovis, NM	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected

Table 2
(*Continued*)

ID[a]	DOF[b]	Origin	BoCV				BRSV				PI3V		
			Gel PCR	Culture	RT-PCR CT	RT-PCR results	Gel PCR	Culture	RT-PCR CT	RT-PCR results	Gel PCR	Culture	Sequence
2208-64	18	East Texas	Positive	Positive	,21.85	Positive	Negative	Negative	,	Not detected	Negative	Positive	PI3V A
2212-39	14	East Texas	Negative	Negative	,20.90	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2213-3	13	East Texas	Negative	Negative	,35.23	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
2218-70	6	Mississsippi	Positive	Positive	,20.22	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-54	6	Mississsippi	Positive	Positive	,23.15	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2198-377	25	Clovis, NM	Negative	Positive	,36.38	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
2163-251	94	Mississsippi	Positive	Positive	,17.12	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2203-442	24	Mississsippi	Negative	Negative	,29.52	Positive	Negative	Negative	,	Not detected	Negative	Negative	
No tag	NA[d]	No info	Negative	Negative	,30.82	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2205-449	25	Mississsippi	Negative	Negative	,33.01	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2205-441	25	Mississsippi	Negative	Negative	,31.55	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2209-426	17	Mississsippi	Positive	Positive	,18.09	Positive	Negative	Negative	,33.72	Positive	Negative	Negative	
2212-30	15	East Texas	Negative	Negative	,29.72	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2213-14	14	East Texas	Negative	Negative	,29.34	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2214-10	NA	East Texas	Positive	Positive	,19.79	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2217-10	5	Mississsippi	Positive	Positive	,23.05	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2217-8	5	Mississsippi	Positive	Negative	,27.65	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2217-31	5	Mississsippi	Negative	Negative	,24.15	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2217-32	5	Mississsippi	Negative	Negative	,20.87	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-93	5	Mississsippi	Negative	Positive	,23.50	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-9	5	Mississsippi	Negative	Negative	,31.83	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2219-34	2	Clovis, NM	Negative	Negative	,34.52	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2219-11	2	Clovis, NM	Negative	Negative	,27.45	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2143-392	99	Mississsippi	Negative	Negative	,34.96	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2206-336	26	Mississsippi	Negative	Negative	,29.12	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2209-437	20	Mississsippi	Negative	Negative	,27.80	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2209-455	20	Mississsippi	Negative	Negative	,30.72	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-51	7	Mississsippi	Positive	Negative	,22.27	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-74	7	Mississsippi	Negative	Negative	,31.02	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-20	7	Mississsippi	Positive	Negative	,22.63	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2219-17	4	Clovis, NM	Positive	Negative	,22.98	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2220-487	4	Clovis, NM	Negative	Negative	,34.59	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2209-436	21	Mississsippi	Negative	Negative	,30.84	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2212-24	18	East Texas	Positive	Negative	,23.76	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2212-1	18	East Texas	Negative	Negative	,31.86	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2212-38	18	East Texas	Positive	Negative	,26.21	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2217-18	8	Mississsippi	Positive	Negative	,18.09	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-6	8	Mississsippi	Negative	Negative	,29.60	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-42	8	Mississsippi	Positive	Negative	,23.81	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-75	8	Mississsippi	Positive	Negative	,25.81	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-35	8	Mississsippi	Positive	Negative	,27.26	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-18	8	Mississsippi	Positive	Negative	,25.69	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-62	8	Mississsippi	Negative	Negative	,33.63	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-16	8	Mississsippi	Negative	Negative	,30.08	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2218-69	8	Mississsippi	Positive	Negative	,23.61	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2219-38	5	Clovis, NM	Positive	Negative	,18.27	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2219-19	5	Clovis, NM	Positive	Negative	,22.20	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2220-481	5	Clovis, NM	Negative	Negative	,23.25	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2140-254	4	Clovis, NM	Negative	Negative	,33.58	Positive	Negative	Negative	,	Not detected	Negative	Negative	

[a] The animal identification.
[b] Days on feed.
[c] Ct values.
[d] Not available.

Table 3
Summary of virus isolation, PCR testing and viral sequencing from nasal swabs of BRD treated calves recently vaccinated with MLV vaccine – Group 2.

ID[a]	DOF[b]	Temp	Location		BoHV-1					BVDV				
			Feedlot	Vaccine	Gel PCR	Culture	Sequence	q-PCR	q-PCR results	Gel PCR	Culture	Sequence	qRT-PCR	qTR-PCR results
1911-95806	13	105.3	Kansas	Vax 2	Negative	Positive	MLV Vax 1-2	,	Not detected	Negative	Negative		,32.78	Positive
1883-97138	18	105.6	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
1849-98671	29	106.5	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
1858-96614	25	104.9	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
1900-99874	13	104.7	Kansas	Vax 2	Negative	Negative		,35.07[c]	Suspect	Negative	Negative		,36.87	Suspect
194-95779	13	105.2	Kansas	Vax 2	Negative	Negative		,35.35	Suspect	Negative	Negative		,	Not detected
1876-92378	22	105.4	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
1910-99793	12	105.3	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
1900-99545	13	105.3	Kansas	Vax 2	Negative	Negative		,35.83	Suspect	Negative	Negative		,	Not detected
1938-97756	5	104.8	Kansas	Vax 2	Positive	Negative		,31.67	Positive	Negative	Negative		,38.60	Suspect
1927-94130	6	104.6	Kansas	Vax 2	Negative	Negative		,36.17	Suspect	Negative	Negative		,	Not detected
1845-92438	29	104.6	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
1924-95955	10	106.3	Kansas	Vax 2	Positive	Positive	MLV Vax 1-2	,13.62	Positive	Positive	Positive	BVDV1b NCP	,24.75	Positive
1916-92958	11	104.7	Kansas	Vax 2	Negative	Negative		,33.27	Positive	Negative	Negative		,	Not detected
1911-95770	12	107.0	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
1936-93345	5	104.8	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
1911-95812	12	105.2	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
1884-95524	15	105.8	Kansas	Vax 2	Negative	Positive	MLV Vax 1-2	,	Not detected	Negative	Negative		,	Not detected
6160-7679	16	105.1	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,35.60	Suspect
6165-7158	14	102.0	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
7405-4273	21	105.0	Kansas	Vax 2	Positive	Negative		,	Not detected	Negative	Negative		,	Not detected
7750-8609	NA[d]	105.8	Kansas	Vax 2	Negative	Negative		,	Not detected	Negative	Negative		,37.05	Suspect
7405-4594	NA	106.6	Kansas	Vax 2	Negative	Negative		,28.79	Positive	Negative	Negative		,	Not detected
1101-13108157	NA	NA	Kansas	Vax 4	Positive	Negative		,	Not detected	Positive	Positive	MLV BVDV1a C24V	,27.77	Positive
6121-8302	23	105.6	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
6160-7607	17	105.5	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
6127-6579	21	105.0	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
7750-8644	11	106.2	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
7742-7633	18	106.1	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,37.96	Suspect
7738-6225	NA	107.2	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
7749-6569	11	107.0	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Positive	MLV BVDV1a Singer	,32.13	Positive
7415-4710	18	106.0	Kansas	Vax 3	Positive	Positive	MLV Vax 1-2	,27.52	Positive	Negative	Positive	BVDV1b NCP	,28.97	Positive
7437-5511	8	105.0	Kansas	Vax 3	Positive	Positive	MLV Vax 1-2	,33.70	Positive	Negative	Negative		,	Not detected
1101-13108197	NA	NA	Kansas	Vax 3	Positive	Negative		,	Not detected	Positive	Positive	BVDV‘1a Not Vax	,32.17	Positive
7925-563	NA	NA	Texas	Vax 3	Positive	Negative		,	Not detected	Negative	Positive	BVDV1a Not Vax	,30.99	Positive
852-890	14	104.4	Texas	Vax 3	Negative	Positive	Field strain	,	Not detected	Negative	Negative		,36.11	Suspect
876-459	12	106.0	Texas	Vax 3	Positive	Positive	Field strain	,	Not detected	Negative	Positive	BVDV 1b NCP	,28.95	Positive
850-995	20	104.4	Texas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
836-648	25	104.5	Texas	Vax 3	Negative	Positive	Field strain	,	Not detected	Negative	Negative		,	Not detected

Table 3
(*Continued*)

ID[a]	DOF[b]	Temp	Location		BoCV				BRSV				PI3V		
			Feedlot	Vaccine	Gel PCR	Culture	qRT-PCR	qTR-PCR results	Gel PCR	Culture	qRT-PCR	qTR-PCR results	Gel PCR	Culture	Sequence
1911-95806	13	105.3	Kansas	Vax 2	Negative	Negative	,30.88	Positive	Negative	Negative	,	Not detected	Negative	Culture	PI3V B
1883-97138	18	105.6	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Positive	
1849-98671	29	106.5	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
1858-96614	25	104.9	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
1900-99874	13	104.7	Kansas	Vax 2	Negative	Negative	,35.33	Suspect	Positive	Negative	,23.39	Positive	Negative	Negative	
194-95779	13	105.2	Kansas	Vax 2	Negative	Negative	,34.67	Positive	Negative	Negative	,	Not detected	Negative	Negative	
1876-92378	22	105.4	Kansas	Vax 2	Negative	Negative	,34.85	Positive	Negative	Negative	,	Not detected	Negative	Negative	
1910-99793	12	105.3	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
1900-99545	13	105.3	Kansas	Vax 2	Negative	Positive	,31.69	Positive	Positive	Negative	,31.41	Positive	Negative	Negative	
1938-97756	5	104.8	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
1927-94130	6	104.6	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
1845-92438	29	104.6	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
1924-95955	10	106.3	Kansas	Vax 2	Negative	Positive	,32.46	Positive	Positive	Negative	,29.15	Positive	Negative	Negative	
1916-92958	11	104.7	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
1911-95770	12	107.0	Kansas	Vax 2	Negative	Negative	,35.77	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
1936-93345	5	104.8	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
1911-95812	12	105.2	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
1884-95524	15	105.8	Kansas	Vax 2	Negative	Positive	,34.95	Positive	Negative	Negative	,	Not detected	Negative	Negative	PI3V B
6160-7679	16	105.1	Kansas	Vax 2	Negative	Negative	,35.90	Suspect	Negative	Negative	,	Not detected	Negative	Positive	
6165-7158	14	102.0	Kansas	Vax 2	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
7405-4273	21	105.0	Kansas	Vax 2	Negative	Negative	,37.02	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
7750-8609	NA[d]	105.8	Kansas	Vax 2	Positive	Positive	,22.54	Positive	Negative	Negative	,38.74	Suspect	Negative	Negative	
7405-4594	NA	106.6	Kansas	Vax 2	Negative	Negative	,36.31	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
1101-13108157	NA	NA	Kansas	Vax 4	Negative	Negative	,35.34	Suspect	Positive	Negative	,28.80	Positive	Negative	Negative	
6121-8302	23	105.6	Kansas	Vax 3	Negative	Negative	,35.33	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
6160-7607	17	105.5	Kansas	Vax 3	Negative	Negative	,35.33	Suspect	Negative	Negative	,33.71	Positive	Negative	Negative	
6127-6579	21	105.0	Kansas	Vax 3	Negative	Negative	,36.00	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
7750-8644	11	106.2	Kansas	Vax 3	Negative	Negative	,36.07	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
7742-7633	18	106.1	Kansas	Vax 3	Negative	Negative	,31.68	Positive	Positive	Negative	,32.34	Positive	Negative	Negative	
7738-6225	NA	107.2	Kansas	Vax 3	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
7749-6569	11	107.0	Kansas	Vax 3	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
7415-4710	18	106.0	Kansas	Vax 3	Negative	Negative	,31.45	Positive	Positive	Negative	,29.13	Positive	Negative	Negative	
7437-5511	8	105.0	Kansas	Vax 3	Positive	Negative	,23.54	Positive	Negative	Negative	,	Not detected	Negative	Negative	
1101-13108197	NA	NA	Kansas	Vax 3	Negative	Negative	,35.96	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
7925-563	NA	NA	Texas	Vax 3	Positive	Positive	,17.00	Positive	Negative	Negative	,	Not detected	Negative	Negative	
852-890	14	104.4	Texas	Vax 3	Negative	Negative	,33.96	Positive	Negative	Negative	,	Not detected	Negative	Negative	
876-459	12	106.0	Texas	Vax 3	Negative	Negative	,27.40	Positive	Negative	Negative	,	Not detected	Negative	Negative	
850-995	20	104.4	Texas	Vax 3	Negative	Negative	,37.24	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
836-648	25	104.5	Texas	Vax 3	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	

[a] The animal identification.
[b] Days on feed.
[c] Ct values.
[d] Not available.

Table 4
Summary of virus isolation, PCR testing and viral sequencing from nasal swabs of BRD treated calves recently vaccinated with MLV vaccine – Group 3.

ID[a]	DOF[b]	Temp	Location		BoHV-1					BVDV				
			Feedlots	Vaccine	Gel PCR	Culture	Sequence	RT-PCR CT	RT-PCR results	Gel PCR	Culture	Sequence	RT-PCR CT	RT-PCR results
7785-0108	6	104.0	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
7777-8061	20	104.7	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
7777-8064	20	105.2	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
2966-3447	5	107.1	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
2948-2100	21	106.6	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,37.86	Suspect
2966-3329	5	105.2	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
2948-2163	21	104.4	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
2955-1368	5	105.8	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
7491-5976	13	105.2	Kansas	Vax 1	Negative	Positive	MLV Vax 1-2	,33.65[c]	Positive	Negative	Negative		,	Not detected
2968-2015	4	106.3	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
2966-3484	4	105.2	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
6222-7854	17	106.8	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
6229-6864	11	105.4	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
6241-8936	7	105.0	Kansas	Vax 3	Negative	Negative		,	Not detected	Positive	Positive	bvdv1b ncp	,29.13	Positive
4947-810	22	105.0	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
5661-4243	7	105.1	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
5651-8319	15	106.9	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,36.82	Suspect
5654-5797	12	104.4	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
5632-5696	20	104.0	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
4950-778	20	105.5	Kansas	Vax 5	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
4967-333	11	105.3	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
4950-825	20	104.5	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
4953-438	19	104.3	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected
4952-762	20	104.3	Kansas	Vax 3	Negative	Positive	Field strain	,	Not detected	Negative	Negative		,	Not detected
4952-831	20	105.3	Kansas	Vax 3	Negative	Negative		,	Not detected	Positive	Positive	mlv bvdv1a singer	,37.10	Suspect
2948-2122	21	106.2	Kansas	Vax 3	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected

Table 4
(*Continued*)

ID[a]	DOF[b]	Temp	Location		BoCV				BRSV				PI3V		
			Feedlots	Vaccine	Gel PCR	Culture	RT-PCR CT	RT-PCR Results	Gel PCR	Culture	RT-PCR CT	RT-PCR results	Gel PCR	Culture	Sequence
7785-0108	6	104.0	Kansas	Vax 3	Positive	Positive	,22.92	Positive	Negative	Negative	,	Not detected	Negative	Negative	
7777-8061	20	104.7	Kansas	Vax 3	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
7777-8064	20	105.2	Kansas	Vax 3	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
2966-3447	5	107.1	Kansas	Vax 3	Positive	Positive	,17.10	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2948-2100	21	106.6	Kansas	Vax 3	Negative	Negative	,37.38	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
2966-3329	5	105.2	Kansas	Vax 3	Positive	Positive	,16.16	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2948-2163	21	104.4	Kansas	Vax 3	Negative	Negative	,34.36	Positive	Negative	Negative	,	Not detected	Negative	Negative	
2955-1368	5	105.8	Kansas	Vax 3	Negative	Negative	,37.43	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
7491-5976	13	105.2	Kansas	Vax 1	Negative	Negative	,37.43	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
2968-2015	4	106.3	Kansas	Vax 3	Positive	Positive	,21.57	Positive	Positive	Negative	,35.89	Suspect	Negative	Negative	
2966-3484	4	105.2	Kansas	Vax 3	Positive	Negative	,26.42	Positive	Negative	Negative	,	Not detected	Negative	Negative	
6222-7854	17	106.8	Kansas	Vax 3	Negative	Negative	,29.35	Positive	Negative	Negative	,	Not detected	Negative	Negative	
6229-6864	11	105.4	Kansas	Vax 3	Negative	Negative	,37.83	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
6241-8936	7	105.0	Kansas	Vax 3	Negative	Negative	,35.55	Suspect	Negative	Negative	,29.18	Positive	Negative	Negative	
4947-810	22	105.0	Kansas	Vax 3	Negative	Negative	,29.51	Positive	Negative	Negative	,	Not detected	Negative	Negative	
5661-4243	7	105.1	Kansas	Vax 3	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
5651-8319	15	106.9	Kansas	Vax 3	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
5654-5797	12	104.4	Kansas	Vax 3	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Positive	PI3V C
5632-5696	20	104.0	Kansas	Vax 3	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Negative	
4950-778	20	105.5	Kansas	Vax 5	Negative	Positive	,	Not detected	Negative	Negative	,	Not detected	Negative	Positive	PI3V C
4967-333	11	105.3	Kansas	Vax 3	Positive	Negative	,28.67	Positive	Positive	Negative	,	Not detected	Negative	Negative	
4950-825	20	104.5	Kansas	Vax 3	Negative	Negative		Not detected	Negative	Negative	,	Not detected	Negative	Positive	PI3V C
4953-438	19	104.3	Kansas	Vax 3	Negative	Negative	,34.03	Positive	Negative	Negative	,	Not detected	Positive	Positive	PI3V C
4952-762	20	104.3	Kansas	Vax 3	Negative	Negative	,33.12	Positive	Positive	Negative	,25.81	Positive	Negative	Negative	
4952-831	20	105.3	Kansas	Vax 3	Negative	Positive	,32.43	Positive	Negative	Negative	,	Not detected	Negative	Positive	PI3V C
2948-2122	21	106.2	Kansas	Vax 3	Negative	Positive	,37.33	Suspect	Negative	Negative	,	Not detected	Negative	Negative	

[a] The animal identification.
[b] Days on feed.
[c] Ct values.

Table 5
Summary of virus isolation, PCR testing and viral sequencing from tissues of BRD treated calves recently vaccinated with MLV vaccine – necropsy cases from Groups 2 and 3.

ID[a]	DOF[b]	Location		Pathology	Tissue	BoHV-1					BVDV					BoCV				BRSV				PI3V		
		Feedlots	Vaccine	Diagnosis	Homogenate	Gel PCR	Culture	Sequence	q-PCR	q-PCR results	Gel PCR	Culture	Sequence	qRT-PCR	qRT-PCR results	Gel PCR	Culture	qRT-PCR	qRT-PCR results	Gel PCR	Culture	qRT-PCR	qRT-PCR results	Gel PCR	Culture	Sequence
1839-9847	35	Kansas	Vax 2	Severe, fibrinosuppurative and necrotizing tracheitis, compatible with IBR	Trachea	Positive	Positive	Field strain	,23.74[c]	Positive	Negative	Negative		,	Not detected	Negative	Negative	,33.81	Positive	Negative	Negative	,	Not detected	Negative	Negative	
7379-3498	NA[d]	Texas	Vax 3	Gross lesions-tracheitis	Lung	Positive	Positive	MLV Vax 1-2	,29.08	Positive	Negative	Positive	MLV BVDV1a NADL	,39.07	Suspect											
				Fresh tissues only	Trachea	Positive	Positive	MLV Vax 1-2	,25.20	Positive	Negative	Negative				Negative	Negative	,37.43	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
5360-9054	NA	Kansas	Vax 3	Acute to subacute, severe intersititalpneumonia, compatible with AIP	Lung	Negative	Negative		,	Not detected	Negative	Positive	Did not band on PCR	,	Not detected	Negative	Negative	,35.26	Suspect	Negative	Negative	,	Not detected	Negative	Negative	
7399-3408	39	Kansas	Vax 1	Severe fibrinosuppurative tracheitis, compatible with IBR	Trachea	Negative	Negative		,	Not detected	Positive	Positive	Twe bands on PCR	,22.39	Positive	Negative	Positve	,	Not detected	Negative	Negative	,	Not detected	Negative	Positive	PI3V C
				Acute to severe fibrinosuppurative bronchopneumonia and pleuritis												Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative		

6242-7997	7	Kansas	Vax 3	Severe, acute fibrinosuppurative pleuropneumona with lobular necrosis and hemorrhage compatible with pulmonary Mannheimiosis. *Histophilus somni* and *Mannheimia haemolytica* isolated [e]	Lung	Negative	Positive	Field strain	,	Not detected	Positive	Positive	MLV BVDV1a Singer	,29.08	Positive														
7469	5	Kansas	Vax 3	No history, fresh tissues only	Lung	Negative	Negative		,	Not detected	Negative	Negative		,	Not detected	Negative	Positive	,28.04	Positive	Negative	Negative	,	Not detected	Negative			
6250-7254	3	Kansas	Vax 3	Fibrinous pneumonia	Lung	Negative	Positive	Field strain	,	Not detected	Positive	Positive	MLV BVDV1a Singer	,24.96	Positive	Negative	Negative	,	Not detected	Negative	Negative	,	Not detected	Negative	Positive	PI3V B			
																Negative	Negative	,	Not detected	Positive	Negative	,22.74	Positive	Negative	Negative				

[a] The animal identification.
[b] Days on feed.
[c] Ct values.
[d] Not available.
[e] Testing at Kansas State University Veterinary Diagnostic Laboratory, Manhattan, Kansas.

Table 6
Summary of viral identification by various tests.

Study	No.	BoHV1 G-PCR	BoHV1 RT-PCR	BoHV1 Culture	BVDV G-PCR	BVDV RT-PCR	BVDV Culture	BoCV G-PCR	BoCV RT-PCR	BoCV Culture	BRSV G-PCR	BRSV RT-PCR	BRSV Culture	PI3V G-PCR	PI3V Culture
1	49	6	5	3	1	4	3	21	47	9	0	1	0	0	1
2	39	9	6	8	3	8	7	3	13	5	6	7	0	0	2
3	26	0	1	2	2	1	2	6	12	7	3	2	0	1	5
Total	114	15	12	13	6	13	11	30	72	21	9	10	0	1	8
Necropsy	7	2	2	4	3	3	5	0	2	2	1	1	0	0	2

and phylogenetic analysis [31]. In U.S., PI3V vaccine strains contain PI3VA genotype. Phylogenetic analysis of BoCV sequences was based on a section of the S gene protein gene. BoCV clades in the U.S. include BoCV1, 2, and 3 [4].

2.5. Statistical analysis

Comparisons were made using 3×2 contingency table and a chi square test to compare the proportions. If this yielded significant results, then pairs of proportions with 2×2 contingency tables. Significance was expressed as $P < 0.05$.

3. Results

3.1. Vaccination and clinical histories for the feedlot studies and necropsy cases

Vax 1 and 5 contain 5 MLV viruses, BoHV-1, BVDV1a, BVDV2a, PI3V, and BRSV, whereas Vax 2, 3, and 4 contain only three, BoHV-1, BVDV1a, and BVDV2a. Group 1 had 49 animals with 2–99 DOF for sample collection [Table 2]. There were two animals with DOF of 94 or 99. Excluding those two, the DOF ranged from 2–26. Group 2 had 39 animals with DOF of 5–29 [Table 3]. Group 3 had 26 animals with DOF of 4–22 [Table 4]. There were 7 necropsy cases with 3–39 DOF [Table 4]. In these studies (after removing the two animals with over 90 days DOF), DOF of the remainder 119 animals, ranged from 2–39. The mean days on feed before initial collection was 14.9 days.

3.2. Viral identification

Tables 2–5 summarize results for viral culture as well as G-PCR, q-PCR, qRT-PCR for the 114 nasal swabs and 7 necropsy cases. Whereas cultures and G-PCR are given for all 5 viruses, the two real time PCR assays only detected 4 viruses, not PI3V. Ct values for each q-PCR or qRT-PCR positive animal are listed for BoHV-1, BVDV, BoCV, and BRSV. The vaccine/field status is listed. For BVDV, those positive for MLV virus have the subgenotype listed. Similarly most of this information is listed for the necropsy cases along with histopathology in Table 5. Of the 114 nasal swabs and if an animal was positive by at least one test for each virus, 87/114 (76.3%) were positive for any of the targeted viruses (48/49 in Group 1, 21/29 in Group 2, 18/26 in Group 3; total 87/114). All seven necropsy cases had a virus positive test. Using positive results for each type of test and regardless of virus each test detected positives on the 114 nasal swabs: (1) G-PCR (5 viruses), 44/114 (38.6%), q-PCR and qRT-PCR (4 viruses), 81/114 (71.6%), and culture (in MDBK and HRT monolayers), 40/114 (35.1%). The q-PCR and qRT-PCR tests identified more virus positives in nasal swabs than either G-PCR or virus positives in cell cultures (P value = 0.0001). For necropsy cases, 5/7 were positive by a G-PCR test, 5/7 by both real time PCR tests, and all 7 were culture positive. When all tests on nasal swabs were counted and if the animal was positive of at least one of the three tests, there were 14/114 (12.3%) positive for BoHV-1, 14/114 (12.3%) for BVDV, 74/114 (64.9%) for BoCV, 10/114 (8.8%) for BRSV, and 8/114 (7.0%) for PI3V. Adding in positives for each virus in the necropsy cases for a total of 121 cases there were 18/121 (14.9%) positive for BoHV-1, 19/121 (15.7%) positive for BVDV, 76/121 (62.8%) positive for BoCV, 11/121 (9.1%) positive for BRSV, and 10/121 (8.3%) positive for PI3V.

Table 6 summarizes the results based on positive outcome by each test. Of 114 nasal swabs there were 15/114 positive for BoHV-1 by the G-PCR test, 12/114 positive by the q-PCR tests, and 13/114 positive by culture. For BVDV testing of nasal swabs 6/114 were positive by BVDV G-PCR, 13/114 positive by qRT-PCR tests, and 12/114 positive by culture. The qRT-PCR BoCV testing for 114 swabs had 72/114 positive, 30/114 by G-PCR, and 21/114 by culture. There were 47/49 animals in Group 1 positive for BoCV by the qRT-PCR test. For the 121 total samples (114 nasal swabs and 7 necropsy cases, there was an exceptionally large number of qRT-PCR BoCV positives, 74/121 (61.2%) compared to 30/121 (24.8%) for the G-PCR and 21/121 (17.4%) for culture of BoCV. The qRT-PCR test detected greater number of BoCV positives in nasal swabs than either G-PCR or virus culture (P value = 0.0001). For BRSV testing 9/114 were positive by G-PCR, 10/114 by qRT-PCR and none culture positive. The qRT-PCR for PI3V was not available. There was only 1/114 positive for PI3V by the G-PCR and 8/114 positive by culture.

Seven necropsy cases had 2/7 positive for BoHV-1 by G-PCR, 2/7 positive by q-PCR and qRT-PCR, and 4/7 positive by culture. BVDV testing of necropsy cases had 3/7 positive by G-PCR, 3/7 positive by qRT-PCR, and 5/7 positive by culture. BRSV testing found 1/7 positive by G-PCR, 1/7 positive by qRT-PCR and none positive by culture. BoCV testing in necropsy cases, none were positive by G-PCR, however 2/7 were culture positive and two positive by qRT-PCR.

When the relationship of the tests to one another was examined for the 121 samples (114 nasal swabs and 7 necropsy cases), there were numerous cases where an animal may be positive by one test or two tests for a specific pathogen and negative to the one other or two other tests. There were 14 total BoHV-1 positive by q-PCR and 7/14 were positive by both culture and q-PCR. Of the 14 positive BoHV-1 by q-PCR 11/14 were positive by G-PCR. There were 7 BoHV-1 culture positives that were q-PCR negative. There were only 7/121 (5.8%) animals that were positive by all three tests for BoHV-1. For BVDV there were 16 positives by qRT-PCR and 14/16 culture positive. There were 7 positives for G-PCR of those 16 qRT-PCR positives. There were 7/121 (5.8%) cases that were positive for all three BVDV tests. There was one animal that was suspect on qRT-PCR that was positive by G-PCR and two qRT-PCR suspects were culture positive. There were 72 positives for BoCV by qRT-PCR testing with 19/72 culture positive. There were 30/72 qRT-PCT BoCV positives that were G-PCR positive. There were two cases where the qRT-PCR was negative for BoCV with two culture positives. There were only 13/121 (10.7%) cases that were positive by all three BoCV tests. There was one case each that was G-PCR positive but a suspect on qRT-PCR. There were two culture positives for BoCV that were RT-PCR suspects. There were no culture positives for BRSV. There were 11/121 BRSV positives by qRT-PCR with 8/11 positive by the G-PCR. There were 10/121 samples culture positive for PI3V and only one positive by the G-PCR.

3.3. Virus identification by genomic analysis

Virus culture positives were submitted for genetic analysis to determine their specific type, subgenotype, or clade and compared to the vaccine strains (Tables 2–5). There were 18 samples culture positive for BoHV-1 with 11 MLV vaccine strains and 7 field strains, 11/18 (61.1%) as vaccine. There were 17 samples positive for BVDV with 6 MLV vaccine strains, 6/17 (35.3%) as vaccine, and 9 field strains and two that could not be classified. BVDV field strains included 6 BVDV1b NCP strains and three BVDV1a NCP strains unrelated to vaccines. There were two BVDV strains in the necropsy group that could not be identified, one did not band on PCR step for sequencing and the second had two bands on the PCR that were unable to be sequenced. For both BoHV1 and BVDV, those viruses placed in the respective vaccine had greater than 99% up to 100% homology to the respective vaccine strain. There were 23 culture positive BoCV strains with a limited number sequenced for BoCV clade status. There were 8 culture positives from nasal swabs that were sequenced, 4 from Group 1, 1 from Group 2, and 3 from and 2 and all 8 were BoCV clade 2. There were no culture positives for BRSV. There were 10 PI3V culture positives, one calf in Group 1 had PI3V A resulting in 10.0% of the PI3V isolates as vaccine. That calf received MLV vaccine containing PI3V A. Of the remaining 9 PI3V culture positives, three were PI3V B and six were PI3V C indicating field strains.

Whenever vaccine strain was identified with a case, in almost all cases the viruses were the same as the vaccine strain received at processing. There were exceptions: in Group 2 there were two animals (7415-4710 and 7437-5511) receiving Vax 3 at processing, however the BoHV-1 strains identified from nasal swabs were identical to the strains in Vax 1 or Vax 2. For necropsy case, animal 7379-3498 had received Vax 3 at processing; however BoHV-1 identified was identical to the strain in Vax 1 or Vax 2. In addition, that calf had a BVDV strain isolated which was NADL CP strain found in Vax 1 or Vax 2, yet Vax 3 MLV vaccine contains Singer strain. Thus, these isolates appear to be from vaccines given prior to processing. There was no information available for the vaccine status prior to entry. No information on the vaccine administered to cattle in adjacent pens. One necropsy case, 1839-9847 (Table 5) had gross and microscopic lesions of the trachea compatible with the BoHV-1 form (IBR) and with a field strain isolated. However one necropsy case 739-3498 (Table 5) had gross lesions of tracheitis and only fresh tissues were submitted and a vaccine strain was isolated related to the MLV vaccine given at processing.

The DOF from processing to when samples (nasal swabs and necropsy tissues) were collected for recovery of vaccine and field strains for viruses was: (1) BoHV-1, vaccine strains 2–35 days and field strains 3–25 days; (2) BVDV, vaccine strains 3–20 days and field strains 7–18 days; and (3) PI3V, vaccine strain 18 days and field strains 5–20 days. There were no BoCV vaccines reported in the studies. For BoCV, DOF duration from processing till sample collection day was 2–99 days. The DOF duration from processing for the BRSV positives was 3–20 days.

4. Discussion

This study provided new data on respiratory infections of cattle based on variety of several pathogen tests: (1) multiple viruses were recovered using different tests on nasal swabs from cattle pulled for initial BRD treatment after receiving MLV vaccines, and from BRD necropsy cases, (2) three different tests were evaluated for viruses including G-PCR, q-PCR, qRT-PCR, and culture in monolayers, (3) genomic testing was performed on the viruses recovered by cultures to identify the virus as to type, subgenotype, or clade, and (4) it was determined if recovered viruses were vaccinal or field strains.

This study expands the knowledge of the viruses associated with BRD in that the additional characterization of the viruses better identifies the viruses, such as genotype or vaccine origin. Other studies have used virus isolation, fluorescent antibody, immunohistochemistry, and viral culture on bovine samples collected from the respiratory tract of necropsy tissues/swabs [6–12]. Those studies gave excellent information on the etiologic agents found in those materials, however the tests used did not discriminate field versus vaccine strains. With many laboratories having access to molecular sequencing of nucleic acids and analysis, it is more possible that viruses could be identified more completely in the viral isolates or nucleic acids from tissues.

Using multiple tests for the detection of viruses resulted in divergent results. For example, only 10.7% of the samples were positive for BoCV by all three tests, viral culture, G-PCR, and qRT-PCR, and only 5.8% positive by all three tests for BoHV1 and BVDV. There were numerous cases where samples were positive for only one or two tests and negative for the other(s). Potential factors for the differences of identification include: PCR tests detect both viral nucleic acids and infectious virus while viral culture requires more infectious virus than PCR [32]. Additional factors might include individual laboratory testing and interpretation of test results. In this study, three different laboratories performed these three tests. Methods of nucleic acid extraction may vary as well and the potential interference by inhibitors of the PCR tests. An example of the differences in the identification of virus is in this study: 74/121 (61.2%) positive for BoCV by the qRT-PCR, 30/121 (24.8%) by G-PCR, and only 21/121 (17.4%) by viral culture. Of the 74 BoCV positives by qRT-PCR, there were 19 positive by viral culture and 30 positive by G-PCR. The real time PCR testing is a quantitative tests based on CT values and appears more sensitive than the G-PCR. Also the BoCV viral culture only used one passage in culture before testing and more passages may have found more virus positives. Also the level of virus in the nasal swabs for BoCV may not have been high enough for viral culture replication.

This study used samples from clinically ill cattle submitted for testing. Viruses were identified in nasals swabs (114) from cattle pulled for BRD treatments in feedlots. Viruses were found frequently, 87/114 (76.3%) in the initial phases of BRD in the entering cattle, even after MLV vaccinations. Potentially those cattle may have been infected prior to entry or after arrival. Regardless, clinicians and managements submitting samples to diagnostic laboratories and receiving results must interpret these results and determine if management measures are efficacious or modified. The question of whether clinically normal cohort animals were shedding viruses is valid, but was not addressed as the current study dealt with only clinically ill cattle. Recovery rate for each virus from nasal swabs indicates five BRD viruses are common in the initial stages of BRD in feedlots: 14/114 (12.3%) positive for BoHV-1, 14/114 (12.3%) for BVDV, 74/114 (64.9%) for BoCV, 10/114 (8.8%) for BRSV, and 8/114 (7.0%) for PI3V. For 7 necropsy cases, there were positives for each virus: 4/7 BoHV-1, 5/7 BVDV, 1/7 BRSV, and 2/7 PI3V.

Diagnostic laboratories, with the great move to molecular tests such as various PCR tests, usually multiplex, there is less emphasis on virus isolation due to economic factors, volume of samples tested, automation, labor, and speed of testing. However, clinicians seek information on the etiologies of various clinical forms, thus there is greater use of the molecular testing. The multiplex PCR tests offer considerable opportunity for virus identification. These molecular tests detect both infectious virus and viral nucleic acids. Realistically, positive test results give an indication of the presence of the virus, especially on a herd basis. However, there are shortcomings of tests that do not separate vaccine from field

strains such as immunohistochemistry and fluorescent antibody on necropsy tissues and the molecular and even viral culture positives, the reports only indicate the etiological agent without a distinction of field or vaccine strain designation. A recent publication on the traditional and contemporary tests for equine herpesvirus-1 (EHV-1) stated that addition to PCR testing, it is important to perform virus isolation during outbreaks of disease and archive EHV-1 strains for respective molecular characterization and molecular epidemiological investigations [32]. Practically speaking for diagnostic laboratories, should there be evidence of potential legal action regarding the source of infection, saving tissues positive by molecular tests could be archived. Should the requesting clinician or animal owner desire further separation of positives into vaccine or field strains, reference laboratories with more genomic sequencing capabilities might be used for this testing, although there is likely a cost factor.

Regarding the issue of "gold standard for respiratory disease diagnosis" it is important to distinguish infection versus disease [13]. That report looks at the question being asked: organism identification without lesions is confirmation of infection; organism identification plus lesion proves disease (may be clinical or subclinical); organism identification plus lesion plus clinical signs explained by the disease proves clinical disease. [14]. One recommendation to clinicians submitting samples from live animals and the laboratory results positive tests for viruses in the respiratory swabs or peripheral blood from live animals, this is evidence of infection in the herd for group of animals represented by the samples. This should alert the clinician that infectious agents are present, and continual monitoring should include the submission of samples from animals dying of disease, especially those dying of acute disease with clinical signs.

There are divergent opinions on the molecular tests compared to traditional culture and they center on definitions. In the review of EHV-1 diagnostic tests, Balasuriya et al. under detection of virus cited a reference: stated: "Virus isolation in cell culture (gold standard). Virus isolation unequivocally demonstrates the presence of infectious virus in a sample and, as a result, is referred as the "gold standard" for laboratory identification of EHV-1 infection [33]. A somewhat divergent view is expressed in the discussion of virus culture negative/PCR positive results in a feline respiratory infection study [34]. Those authors stated it was incorrect to interpret molecular test results as false positives as they represent true positive results and can be confirmed by either re testing or by sequencing of the PCR product [34].

In the present study, there were limited cases where the animal was positive for all three tests, G-PCR, q-PCR or qRT-PCR, or culture. Those were 7/121 (5.8%) for BoHV-1, 7/121 (5.8%) for BVDV, and 13/121 (10.7%) for BoCV. There were numerous examples where a case scored positive was only positive for one or two of three tests. Thus, selection of the test available may depend on the question being asked, to detect evidence of infection by viral nucleic acid or is recovery of infectious virus more important. Real time-PCR tests, especially multiplex, offers more rapid testing and the Ct values calculated for negative/positive test results. Gel based PCR relies on the subjective visualization of the gel for positive status. In this study, the q-PCR and qRT-PCR detected more viral positive, 71.6%, than the G-PCR, 38.6% or culture. 35.1%. However, there were multiple instances that G-PCR or real time-PCR negative cases had culture positives for BoHV-1, BVDV, BoCV, or PI3V. Therefore, genomic testing alone may not detect these viruses if cell cultures are not used.

Genetic testing by G-PCR, q-PCR, qRT-PCR, and RT-PCR with resulting positives or the culture positives are qualitative in nature and do not specify the strain as field or vaccine strain. Potentially there may be multiple field strains of each of the respiratory tract viruses, and vaccine strains for each virus. Genomic testing by G-PCR, q-PCR and qTR-PCR does not differentiate field strains from vaccine-derived strains. This is borne out as both genomic assays in this study detected both the field and vaccine strains of BoHV-1, PI3V, and BVDV.

There are multiple strains, types, and subgenotypes for respiratory tract viruses and these hold true for both field and vaccine strains. BVDV subgenotypes were then compared to the respective vaccine subgenotypes for identity. In this study finding the subgenotype alone did not specify vaccine strain as there were BVDV1a strains not of vaccine origin. BVDV field and vaccine strains found in this study in culture positives, included 6 MLV 6/17 (35.3%) vaccine strains and 9 field strains. Similarly BoHV-1 culture isolates were analyzed with sequence information permitting assignment of the strain specific to a vaccine group or to field strain status with 11 MLV vaccine strains 11/18 (61.1%) and 7 field strains. The same was observed for PI3V with the PI3V a type of potential vaccine status (1 isolate) compared to PI3V B and PI3V C considered as field strains (9 strains) [31]. Thus in this study there were both vaccine and field strains for BoHV-1, BVDV, and PI3V detected, but only after the positive culture samples were analyzed by further genetic sequencing. The primers used in this study for any of the viruses or test did not permit separation of the positive viruses into vaccine or field strain status.

Recovery of MLV vaccine strains from cattle either in ante mortem testing such as respiratory tract samples or peripheral blood or from necropsy specimen is not unexpected. MLV strains may replicate in the host. Replicating viruses have potential to spread within the host as evidenced by BVDV and BoHV-1 in certain vaccines to cross the placenta resulting in fetal infections [1]. Prior studies have reported several different BoHV-1 MLV vaccine strains in respiratory tract samples from BRD cases [20,30]. BVDV vaccine strains were cultured from lungs of cattle dying in feedlots [6]. In other species, the effects of vaccination on viral detection have been varied. In the review of the EHV-1 cited prior, the authors reported that specific studies had not been conducted to determine if the MLV vaccine strain of EHV-1 is detectable in nasopharyngeal or nasal swabs following vaccination [33]. However, a reference was cited where the MLV vaccine strain has been identified in aborted fetuses [35] There has not been exhaustive studies taking samples up to days after vaccination for the parenteral administered MLV vaccines. One study administered a MLV vaccine containing BoHV1, BVDV1a and BVDV2a to seronegative calves. Unvaccinated control calves (in a separate pen) were included along with pregnant cows housed with vaccinates [36]. Nasal swabs and peripheral blood was collected through day 42. The nasal swabs and blood were tested using virus culture in bovine monolayers and a gel-based PCR test. Serums of vaccinates and controls were tested for viral antibodies to BoHV1, BVDV1, and BVDV2. All nasal swabs were negative for BoHV1 and BVDV in all collections. The buffy coat samples of vaccinates were negative except for 9/18 vaccinates BVDV positive on day 7 or 10 only while all other collections were negative. All blood samples were negative for BoHV1. All nasal swab collections were negative by gel-based PCR for BoHV1 and BVDV. There were PCR positives in blood samples 14/18 vaccinates for BVDV at days 3, 7, or 10 after vaccination. Control calves and contacts were negative by virus isolation and PCR in all collections. The control calves and contacts with vaccinates remained seronegative though out the study. While a transient viremia (cell culture positive) and blood positive by PCR occurred after vaccination, there was no evidence of viral shedding [36]. If virus is found in MLV vaccinates, the detection of MLV strains in calves' nasal secretions receiving parenteral vaccines by label route does not mean calves are capable of infecting other animals. Nasal swabs had recovered infectious viruses using qualitative tests that did not quantitate the virus amount. There is likely a quantitative threshold of virus required in nasal secretions to infect other animals.

The lack of recovery of BRSV via cell culture isolation was not unexpected. Studies using ELISA, PCR test and virus isolation in cell culture was performed in samples experimentally infected with BRSV [37]. The PCR test and to a lesser extent, the ELISA, may detect virus shedding for a longer period after infection than virus isolation. Simulated environmental conditions likely to be experienced during transport of clinical field specimens markedly reduced the sensitivity of virus isolation but had minimal effect on the results of ELISA. Actual field transport conditions (overnight on ice) had minimal apparent effect on the results of the PCR assay [37].

5. Conclusions

This study identified viruses in cattle pulled BRD treatment shortly after arrival in the feedlot, and after the cattle received MLV viral vaccine at processing. There were necropsy cases of varied BRD. Nasal swabs and tissue homogenates were tested by gel-based PCR (G-PCR), real time PCR (q-PCR or qRT-PCR) and cell cultures. Recovery of viruses in the nasal swabs of 114 cattle and the 7 necropsy cases was common: 76.3% of the nasal swabs were positive for virus in at least one of the three tests, and all 7 necropsy cases were positive for at least one virus. The recovery rate for each virus from nasal swabs indicated five viruses associated with BRD are common shortly after arrival. RT-PCR tests detected more positive cases in nasal swabs than G-PCR and cultures, however, one study had an extreme large numbers of BoCV qRT-PCR positives compared to other tests. Numerous cases were positive for only one or two tests and negative for other(s). There were cases where genomic tests did not detect BoHV-1, BVDV, BoCV, or PI3V culture positives. Field and vaccine strains of BoHV-1, BVDV, and PI3V were recovered by culture. PCR tests did not differentiate field strains from vaccine strains for BoHV-1, BVDV, and PI3V. Additional testing including genomic sequencing/phylogenetic analysis or using specific primers and sequencing to delineate vaccine from field strains is needed.

Acknowledgements

This research was supported by the Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK via the research support from the Endowed Chair, McCasland Foundation for Food Animal Research support of Dr. R. Fulton. The Wentz Scholars program and the Niblack Research program supported the undergraduate research of Ms. Casey Landis.

Conflict of interest statement: The authors have no consulting or financial interest relationship with the funding agency.

References

[1] Fulton RW. Viral diseases of bovine respiratory tract: bovine herpesvirus-1, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine adenoviruses, bovine coronavirus, and bovine viral diarrhea viruses. In: Anderson DE, Rings DM, editors. Current veterinary therapy – food animal practice. Fifth volume. 2008. p. 171–91 [chapter 42].
[2] Fulton RW. Bovine respiratory disease research: 1983–2009. Anim Health Res Rev 2009;10:131–40.
[3] Fulton RW, Step DL, Wahrmund J, Burge LJ, Payton ME, Cook BJ, et al. Bovine coronavirus (BCV) infections in transported and commingled beef cattle and sole-source calves. Can J Vet Res 2011;75:191–9.
[4] Fulton RW, Ridpath JF, Burge LJ. Bovine coronaviruses from the respiratory tract: antigenic and genetic diversity. Vaccine 2013;31:886–92.
[5] Compendium of veterinary products. 12th ed. Port Huron, MI: North American Compendiums; 2010. p. 1–1848.
[6] Fulton RW, Blood KS, Panciera RJ, Payton ME, Ridpath JF, Confer AW, et al. Lung pathology and infectious agents in fatal feedlot pneumonias and relationship with mortality, disease onset, and treatments. J Vet Diagn Invest 2009;21:464–77.
[7] Gagea MI, Bateman KG, van Dreumel T, McEwen BJ, Carman S, Archambault M, et al. Diseases and pathogens associated with mortality in Ontario beef feedlots. J Vet Diagn Invest 2006;18:18–28.
[8] Booker CW, Abutarbush SM, Morley PS, Jim GK, Pittman Tj, Schunicht OC, et al. Microbiological and histopathological findings in cases of fatal bovine respiratory diseases in feedlot cattle in western Canada. Can Vet J 2008;49:473–81.
[9] Moore SJ, O'Dea MA, Perkins N, Barnes A, O'Hara AJ. Mortality of live export cattle on long-haul voyages: pathologic changes and pathogens. J Vet Diagn Invest 2014;26:253–65.
[10] Klima CL, Zaheer R, Cook SR, Booker CW, Hendrick S, Alexander TW, et al. Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative elements. J Clin Microbiol 2014;52:438–48.
[11] Horwood PF, Mahoney TJ. Multiplex real-time RT-PCR detection of three viruses associated with bovine respiratory disease complex. J Virol Methods 2011;171:360–3.
[12] Moore SJ, O'Dea MA, Perkins N, O'Hara AJ. Estimation of nasal shedding and seroprevalence of organisms known to be associated with bovine respiratory disease in Australian export cattle. J Vet Diagn Invest 2015;27:6–17.
[13] Fulton RW, Confer AW. Laboratory test descriptions for bovine respiratory disease diagnosis and their strengths and weaknesses: gold standards for diagnosis, do they exist. Can Vet J 2012;53:754–61.
[14] Hierhozer JC, Killington RA. Virus isolation and quantitation. In: Mahy BWJ, Kangro HO, editors. Virology methods manual. London: Academic Press; 1996.
[15] Fulton RW, Whitley EM, Johnson BJ, Ridpath JF, Kapil S, Burge LJ, et al. Prevalence of bovine viral diarrhea virus (BVDV) in persistently infected cattle and BVDV subtypes in affected cattle in beef herds in South Central United States. Can J Vet Res 2009;73:283–91.
[16] Fulton RW, Hessman B, Johnson BJ, Ridpath JF, Saliki J, Burge LJ, et al. Evaluation of diagnostic tests used for detection of bovine viral diarrhea virus and prevalence of BVDV subtypes 1a, 1b, and 2a. In persistently infected cattle entering a feedlot. J Am Vet Med Assoc 2006;228:578–84.
[17] Fulton RW, Hessman BE, Ridpath JF, Johnson BJ, Burge LJ, Braziel B, et al. Multiple diagnostic tests to identify cattle with bovine viral diarrhea virus and duration of positive tests in persistently infected cattle. Can J Vet Res 2009;73:117–24.
[18] Fulton RW, Saliki JT, Confer AW, Burge LJ, d'Offay JM, Helman RG, et al. Bovine viral diarrhea virus cytopathic and noncytopathic biotypes and type 1 and 2 genotypes in diagnostic laboratory accessions: clinical and necropsy samples from cattle. J Vet Diagn Invest 2000;12:33–8.
[19] d'Offay JM, Fulton RW, Eberle R. Complete genome sequence of the NVSL BoHV-1.1 Cooper Reference Strain. Arch Viral 2013;158:1109–13.
[20] Fulton RW, d'Offay JM, Eberle R, Moeller RB, Van Campen H, O'Toole D, et al. Bovine herpesvirus-1: evaluation of genetic diversity of subtypes derived from field strains of varied clinical syndromes and their relationship to vaccine strains. Vaccine 2015;33:549–58.
[21] Lyon M, Leroux C, Greenland T, Chastang J, Patet J, Mornex JF. Presence of a unique parainfluenza virus 3 strain identified by RT-PCR in Visna-Maedi Infected Sheep. Vet Microbiol 1997;51:95–104.
[22] Dee S, Neill C, Clement T, Christopher-Jennings J, Nelson E. An evaluation of a liquid antimicrobial (Sal CURB) for reducing the risk of porcine epidemic diarrhea virus infection of naïve pigs during consumption of contaminated feed. BMC Vet Res 2014;10:1–6.
[23] Mahlum C, Haugerud S, Shivers JL, Rossow KD, Goyal SM, Collins JE, et al. Detection of bovine viral diarrhea virus by taqman reverse transcriptase chain reaction. J Vet Diagn Invest 2002;14:120–5.
[24] Brower A, Homb KM, Bochsler P, Porter R, Woods K, Ubi S, et al. Toohey-Kurth. Encephalitis in aborted fetuses associated with BHV-1 infection. J Vet Diagn Invest 2008;20:297–303.
[25] Moore S, Gunn M, Walls D. A rapid and sensitive PCR-based diagnostic assay to detect BHV-1 in routine diagnostic submissions. Vet Microbiol 2000;75:145–53.
[26] Boxus M, Letellier C, Kerkhofs P. Real-time RT-PCR for the detection and quantification of BRSV. J Virol Methods 2005;125:125–30.
[27] Liu L, Hagglund S, Hakhverdyan M, Alenius S, Larsen LE, Belak S. Molecular epidemiology of BCV on the basis of comparative analysis of the S Gene. J Clin Microbiol 2006;44:957–60.
[28] Hasoksuz M, Sreevatsan S, Cho KO, Hoet AE, Saif LJ. Molecular analysis of the S1 subunit of the spike glycoprotein of respiratory and enteric bovine coronavirus isolates. Virus Res 2002;84:101–9.
[29] Fulton RW. Impact of species and subgenotypes of bovine viral diarrhea virus on control by vaccination. Anim Health Res Rev 2015;16:40–54.
[30] Fulton RW, d'Offay JM, Eberle R. Bovine herpesvirus-1: comparison and differentiation of vaccine and field strains based on genomic sequence variation. Vaccine 2013;31:1471–9.
[31] Neill JD, Ridpath JF, Valayudhan BT. Identification and genome characterization of genotype B and genotype C bovine parainfluenza type 3 viruses isolated in the United States. BMC Vet Res 2015;11:112.
[32] Balasuriya UB, Crossley BM, Timoney PJ. A review of traditional and contemporary assays for direct and indirect detection of Equid herpesvirus 1 in clinical samples. J Vet Diagn Invest 2015;27:687.
[33] World Health Organization for Animal Health (OIE). Equine rhinopneumonitis. In: OIE manual of diagnostic tests and vaccines for terrestrial

animals (mammals, birds, and bees). 7th ed. Paris, France: OIE; 2012 [chapter2.5.9].
[34] Litster A, Wu CC, Leutenegger CM. Detection of feline upper respiratory tract disease pathogens using a commercially available real-time PCR test. Vet J 2015;206:149–53.
[35] Smith KL, Allen GP, Branscum AJ, Cook RF, Vickers ML, Timoney PJ, et al. The increased prevalence of neuropathogenic strains of EHV-1 in equine abortions. Vet Microbiol 2010;141:5–11.
[36] Kleiboeker SR, Lee SM, Jones CA, Estes M. Evaluation of shedding of bovine herpesvirus-1, bovine viral diarrhea virus 1, and bovine viral diarrhea virus 2 after vaccination with a multivalent modified-live virus vaccine. J Am Vet Med Assoc 2003;222:1399–403.
[37] West K, Bogdan J, Hamel A, Nayar G, Morley PS, Haines DM, et al. A comparison of diagnostic methods for the detection of bovine respiratory syncytial virus in experimental clinical specimens. Can J Vet Res 1998;62:245–50.