

BRIEF RESEARCH REPORTS

Detection of all eight serotypes of *Epizootic hemorrhagic disease virus* by real-time reverse transcription polymerase chain reaction

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Abstract. *Epizootic hemorrhagic disease virus* (EHDV) has been associated with bluetongue-like disease in cattle. Although U.S. EHDV strains have not been experimentally proven to cause disease in cattle, there is serologic evidence of infection. Differentiation of *Bluetongue virus* (BTV) and EHDV is necessary because diagnosis of infection caused by these viruses is often confused. The previously developed nested reverse transcription polymerase chain reaction (nRT-PCR) test for indigenous EHDV disease is sensitive and specific, but it is prone to contamination problems. Additionally, the EHDV nRT-PCR only detects 7 of the 8 serotypes. To develop an improved diagnostic test, sequence analysis was performed on 2 conserved target genes; one is highly expressed in infected mammalian cells, whereas the other is highly expressed in infected insect cells. This information was used to develop a rapid EHDV real-time PCR that detects all 8 EHDV serotypes. The EHDV assay did not cross-react with BTV strains and performed similarly to the nRT-PCR tests with archived clinical samples. In addition, it is superior to the nRT-PCR, not only because it is a closed system with fewer cross-contamination problems, but also because it detects all 8 serotypes and is less labor and time intensive.

Key words: *Bluetongue virus*; *Epizootic hemorrhagic disease virus*; real-time reverse transcription polymerase chain reaction.

Epizootic hemorrhagic disease virus (EHDV; family *Reoviridae*, genus *Orbivirus*) is an insect-transmitted pathogen of ruminants causing periodic significant losses in wild and captive deer populations.²¹ *Epizootic hemorrhagic disease virus* is closely related to *Bluetongue virus* (BTV), which can also cause hemorrhagic disease in deer.¹¹ The seroprevalence of BTV and EHDV ranges from 45–100% in deer populations.^{6,8} BTV causes an estimated \$120 million in annual loss to the U.S. livestock industry and about \$3 billion in annual losses worldwide (Bath GF: 1989, *Bluetongue*. In: *Proceedings of the 2nd International Congress for Sheep Veterinarians*, pp. 349–357). A clinically similar disease caused by BTV has been associated with EHDV infection in cattle^{4,9}; however, U.S. serotypes of EHDV have not been shown to cause clinical disease in experimental infections.¹ There is also serologic evidence of infection in and isolation of EHDV from U.S. cattle and sheep.^{14,23} Therefore, rapid diagnosis and differentiation of BTV and EHDV are needed for both livestock and wildlife.

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An understanding of the molecular attributes of these viruses is necessary to develop effective gene-based diagnostic tests. The genome for these viruses comprises 10 segments of double-stranded RNA, which encode for 7 structural and 4 nonstructural proteins.^{13,24} There are 24 serotypes of BTV and 8 confirmed serotypes of EHDV.^{11,25} Serotype is determined primarily by the outer capsid protein VP2, but it can also be influenced by the second outer capsid protein VP5.^{17,29} The inner core proteins VP3 and VP7 are fairly conserved within the virus serogroup.^{15,27,31} Nonserotype-specific enzyme-linked immunosorbent assays (ELISAs) for these viruses are usually based on the VP7 protein.¹⁶ Nonstructural proteins of EHDV are highly conserved among North American viruses.^{18,19,28} This information has been used to develop rapid genetic amplification tests for early detection and differentiation of indigenous strains of EHDV.^{2,28} These previously developed assays all required gel electrophoresis for amplicon detection; therefore, they are time consuming and prone to sample contamination problems. To avoid these problems, a previously described real-time reverse transcription polymerase chain reaction (real-time RT-PCR) assay was developed for EHDV based on sequence data available for the 2 U.S. serotypes of EHDV.³² However, this assay did not detect all 8 known serotypes of EHDV.

The recent isolations of an exotic serotype of EHDV in the United States (Pearson JE, Wilson WC, Barber TL, et al.: 2007, Report of the Committee on Bluetongue and Bovine Retroviruses. U.S. Animal Health Association. Available at <http://www.usaha.org/committees/reports/>

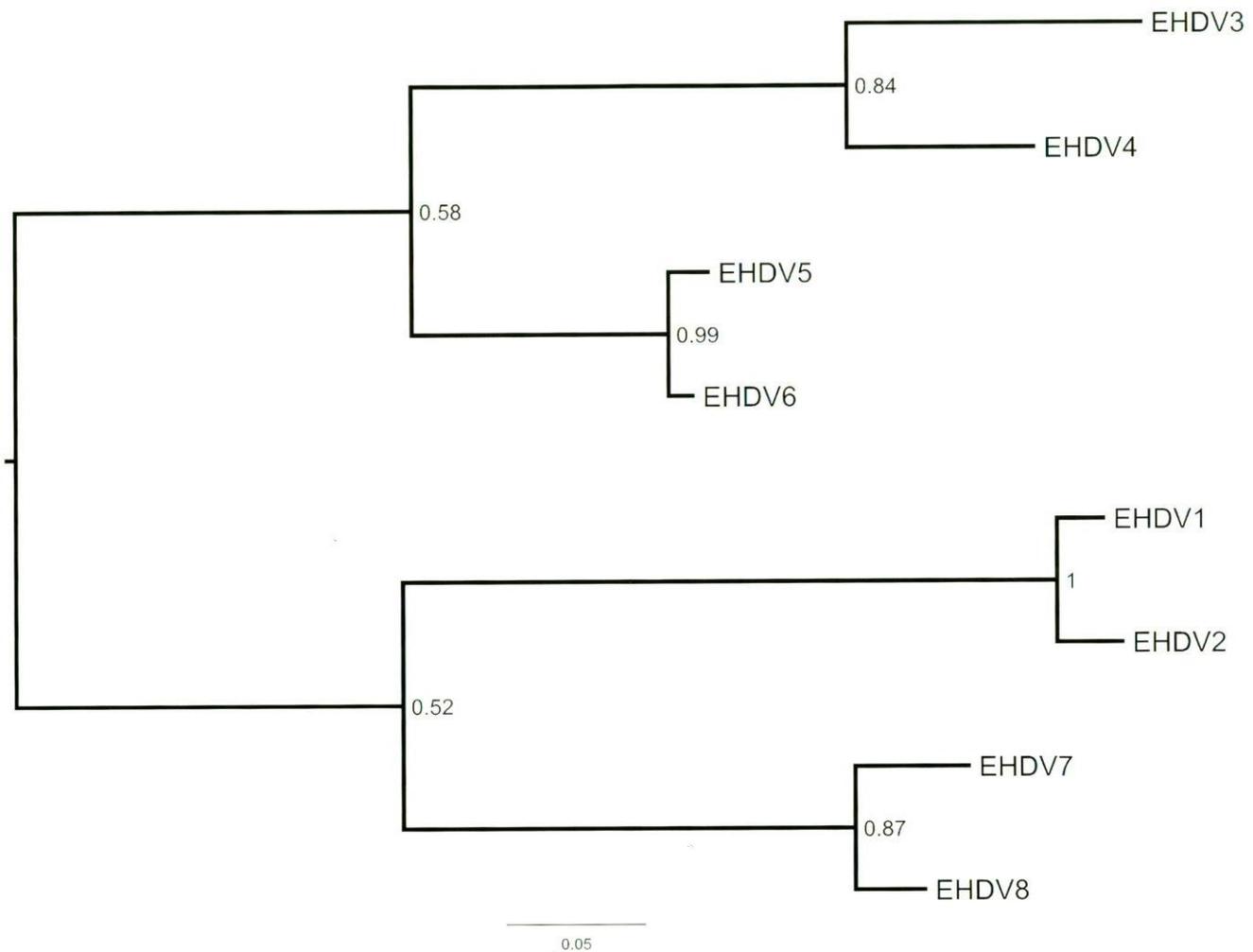


Figure 1. Phylogenetic analysis of the M5 gene from prototype *Epizootic hemorrhagic disease virus* (EHDV) serotypes. Sequences were aligned using MUSCLE, and the tree was calculated using MrBayes (750,000 generations, GRT+ Γ +Inv). Numbers on nodes indicate posterior probabilities. Branch lengths are in nucleotide changes per site.

2007/report-btbr-2007.pdf. Accessed November 21, 2008) clearly demonstrate the need for an improved assay that rapidly detects all known strains. Two genes that are conserved among U.S. serotypes^{10,28} were chosen as target amplicons. One gene is highly expressed in infected mammalian cells, segment M5, which encodes nonstructural protein 1 (NS1),²⁴ and the other is highly expressed in infected insect cells, segment S10, which encodes nonstructural protein 3 (NS3).⁵ Sequence analysis of the remaining EHDV serotypes provided the foundation for developing a more robust diagnostic test.

The EHDV-3 through EHDV-8 prototype viruses were kindly provided by the National Veterinary Services Laboratories. Total RNA was extracted from cells using a commercial extraction kit^a per the manufacturer's protocol. Ethanol precipitation was omitted, and the double-stranded RNA (dsRNA) was purified using lithium chloride differential precipitation as previously described.²⁶ Amplification and sequencing using standard protocols^d of the M5 and S10 genes from the exotic EHDV prototype strains were performed as previously described.^{3,29} The

primer sequences, except for the first 5 bases on the 5' end that included the ATG start codon for the S10, were removed for data analysis and reported to GenBank (accession nos. EU928893–EU928904). The EHDV M5 gene had a percent identity range from 77.7% (serotypes 4–6 or 7) to 98.6% (serotypes 1–2). The EHDV S10 gene had a percent identity range from 77.2% (serotypes 1–3) to 96.5% (serotypes 1–2). Full-length sequences were aligned using either ClustalX²² or MUSCLE.⁷ Phylogenetic trees were calculated from these multiple sequence alignments using MrBayes²⁰ using the standard nucleotide substitution model (4×4) with GTR (nst = 6). Substitution rates were set to invgamma (gamma-shaped rate variation with a proportion of invariable sites). Default values were used for all other settings. A total of 750,000 generations were calculated by sampling every 100th tree, and a consensus tree was calculated after a burn-in of 2,500 trees using the allcompat setting. The EHDV M5 separated into 2 major lineages with a very high degree of confidence. Phylogenetic analyses of M5 and S10 were performed using several algorithms showing similar profiles regardless of the

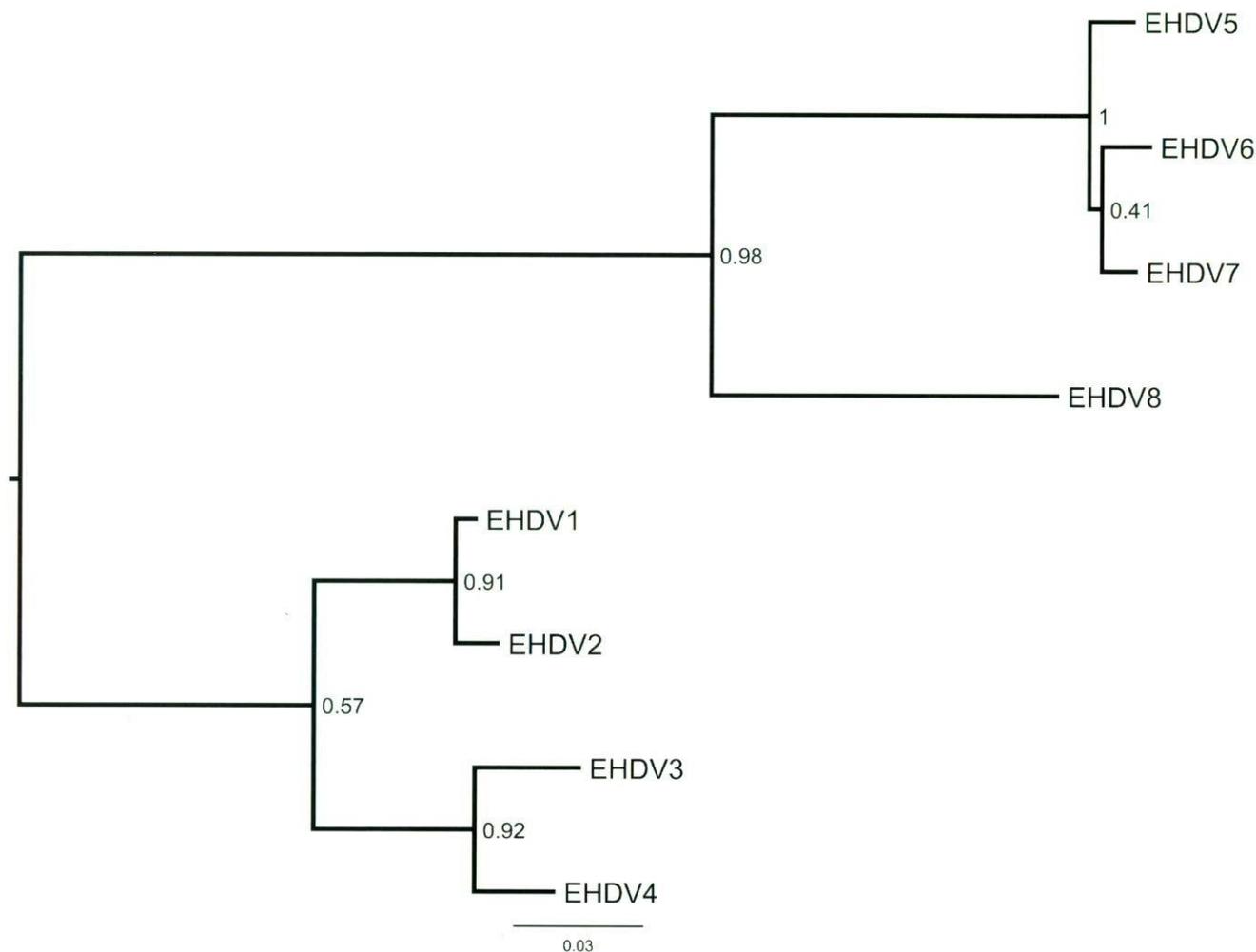


Figure 2. Phylogenetic analysis of the S10 gene from prototype *Epizootic hemorrhagic disease virus* (EHDV) serotypes. Sequences were aligned using MUSCLE, and the tree was calculated using MrBayes (750,000 generations, GRT+I+Inv). Numbers on nodes indicate posterior probabilities. Branch lengths are in nucleotide changes per site.

algorithm but distinct by gene segment (Figs. 1 and 2, respectively).

Surprisingly, after close examination of the M5 sequence alignment, conserved regions between all the serotypes of sufficient size to develop a real-time PCR primer set were not found. Therefore, the authors' attention turned to the second target for the primer design. Sequence analysis of the S10 gene from North American EHDV prototype and U.S. field strains showed 94.9–100% identity.^{10,18} The S10 also had a greater diversity than expected; however, a region was identified to be suitably conserved to be used to design primers using Primer Express v. 2.0 software.^c The primers were initially tested using SYBR Green RT-PCR^c to verify amplification. Probes were labeled with a 5' FAM reporter and 3' BHQ-1 quencher.^f For optimal detection of all 8 EHDV serotypes, the real-time RT-PCR assay used 7 primer sets and 3 probes. Probe 1/2 detected EHDV serotypes 1, 2, 5, 6, 7, and 8. Probe 3 detected EHDV serotype 3, and probe 4 detected EHDV serotype 4. Each serotype had its own amplification primer pair except serotypes 1 and 2, which shared a primer set (1/2; Table 1).

TaqMan EZ-RT PCR reagents^e were used for the real-time RT-PCR in half-reaction volumes and included 0.4 μ mol of each primer and 0.08 μ mol of each probe. The cycling parameters were based on the previously reported real-time RT-PCR for *Vesicular stomatitis virus*.³⁰ The cycle temperatures were 55°C for 25 min, 95°C for 2 min, 40 cycles of 95°C for 10 sec, and 55°C for 1 min. The samples were analyzed using 3 real-time PCR instruments.^{c,g,h} All 3 instruments performed similarly, and use was determined based on instrument availability and number of samples. Analysis of the limit of detection (LOD) was performed using dsRNA from all 8 serotypes of EHDV and was done in duplicates using 10-fold serial dilutions. The EHDV serotypes 1 and 2 using a single probe detected down to at least 2 fg (~100 RNA copies). The assay using all 3 probes detected EHDV serotype 1 at 20 fg (~1,000 RNA copies), serotypes 2 and 6 to at least 2 fg, serotypes 3 and 5 at 200 fg (~10,000 RNA copies), and serotypes 7 and 8 at 2 pg (~100 RNA copies). The sensitivity of the assay did not increase by the addition of a heat denaturant step. A 10-fold increase in sensitivity with

Table 1. Epizootic hemorrhagic disease virus (EHDV) real-time primer and probe sequences.

Serotype	Forward	S10*	Probe	S10	Reverse	S10	Length
EHD1/2	GGTTGGATATTTGGACAAAGC	165-187	TCAAATCAAACGGGGCAACTATGG	192-216	GCATACGAAGCATAAGCAACCTT	275-253	110
EHD3	AGCCCTCGACATTCGGATAAG	164-185	CAAATCAAACCTGGTGCCACGATGGC	206-230	CGGACTTTCACACTTTTGG	259-239	95
EHD4	AAGTTGCCCTCGATATCCTAGATAAG	160-185	CAATGCTAACAACAGGGCTACAATGG	199-228	GTAAGGACCTTTTACCTTTTGA	266-242	106
EHD5	GCTGGATATACTCGACAAAGCAATG	167-191	TCAAATCAAACGGGGCAACTATGG	192-216	ACGCGACCTTTTACTCTTTTGA	262-240	95
EHD6	GAGTCGGCTGGATATACCTC	160-179	TCAAATCAAACGGGGCAACTATGG	192-216	GCATATGATGCATACGGGACCTTT	276-253	97
EHD7	CAAAAGTTGCCCTTGACATTTTAG	157-180	TCAAATCAAACGGGGCAACTATGG	192-216	CATATGCTACTTTTCTACCTCTCGC	266-243	109
EHD8	CCAAAAGTCGCCCTTGATATT	156-177	TCAAATCAAACGGGGCAACTATGG	192-216	CATATGCCACTTTTCTACCTCTGCG	268-243	112

* Numbers indicate nucleotide position in the S10 gene.

methyl mercury hydroxide denaturation in the domestic BTV or EHDV real-time RT-PCR assays has been previously reported³²; however, this reagent is no longer commercially available. Other chemical denaturants were not as effective in improving sensitivity and reduced the amount of input template, and thus deemed not sufficiently advantageous to add a denaturation step.

The LOD determination is only an estimate based on purified dsRNA input. To better evaluate the assay performance, the real-time assay was compared to the nested RT-PCR (nRT-PCR)²⁸ on archived clinical material. In addition, available samples were analyzed by virus isolation (VI) and were also confirmed by an antigen-capture ELISA.^{12,16} Total RNAs from clinical samples were extracted using a commercial kit,^b with minor modifications. The PCR assays were tested on 44 U.S. EHDV isolates, 36 tissue or blood samples from pronghorn antelope, white-tailed deer, and mule deer, and 23 archived clinical RNA samples, for 103 total samples. The U.S. isolates and clinical samples were mostly detected by the real-time RT-PCR assay at 200 pg of total RNA; however, 6 field strains and 8 clinical samples required a higher concentration. All 44 EHDV U.S. isolates tested positive by both real-time RT-PCR and nRT-PCR. Of the clinical samples, 19 tested positive by both real-time RT-PCR and nRT-PCR (Table 2). Additionally, this real-time RT-PCR assay was run against all 24 BTV serotypes to analyze cross-reactivity, and all were found to be negative.

Thirty-six of the archived clinical samples were tested by standard VI and antigen-capture ELISA to confirm the presence of EHDV.¹⁶ Of VI-positive samples, 10 were positive by EHDV antigen-capture ELISA, for a prevalence of 28%. Two of the EHDV VI-negative samples were negative for EHDV by both nRT-PCR and real-time RT-PCR and were found positive by BTV antigen-capture ELISA.¹² One VI-positive sample contained both BTV and EHDV antigens as detected by the appropriate antigen-capture ELISAs. This sample was also positive for EHDV by both RT-PCR assays. Nine samples were VI negative but were positive for EHDV RNA by both RT-PCR assays. The real-time RT-PCR performed identically to the nRT-PCR with the 24 archived clinical RNA samples (Table 2).

The initial single-probe design only detected EHDV serotypes 1, 2, 5, 6, 7, and 8. Attempts to detect EHDV serotypes 3 and 4 using degenerative probes and modifying the protocol were unsuccessful. Additional probes were necessary for detection of these 2 serotypes. Thus, optimal detection of all 8 serotypes included the design of 7 amplification primers and 3 detection/confirmation probes. This EHDV all-serotype detection assay did not cross-react with any of the 24 BTV serotypes. This preliminary assay in the current study is based solely on the EHDV S10 target, but it performs identically to a previous EHDV nRT-PCR²⁸ that was designed solely for North American serotypes. This assay is an improvement over the previous assay in that it is a closed system (and thus has fewer cross-contamination problems), is less time consuming than traditional nRT-PCR, does not require a toxic methyl mercury denaturation step, and detects all 8 EHDV serotypes. Given the recent detection of exotic EHDV

Table 2. Epizootic hemorrhagic disease virus (EHDV) real-time reverse transcription polymerase chain reaction (real-time RT-PCR) compared with EHDV nested RT-PCR and EHDV virus isolation (VI) on various samples.*

	<i>n</i>	Real-time RT-PCR ⁺	Nested RT-PCR ⁺	Real-time RT-PCR ^{+/VI} ⁺	Real-time RT-PCR ^{-/VI} ⁻	Real-time RT-PCR ^{-/VI} ⁺	Real-time RT-PCR ^{+/VI} ⁻
Clinical samples	36	19	19	10	17	0	9
Archived RNA	23	4	4	NA	NA	NA	NA
U.S. EHDV isolates	44	44	44	NA	NA	NA	NA
Bluetongue virus isolates	24	0	0	NA	NA	NA	NA

* + = positive; - = negative; NA = not applicable.

serotype 6 in the United States, this should prove to be a useful assay for veterinary diagnostic laboratories. Recently, increased sensitivity has been achieved using an alternative RNA extraction and detection method for use in a multiplex BTV/EHDV real-time RT-PCR assay (Wilson, Hindson, O'Hearn, et al., unpublished data). Future improvements will include additional primer sets targeting the M5 gene to provide further assay assurance and also to multiplex using a similar assay currently in development for simultaneous BTV detection.

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Sources and manufacturers

- Purescript[®] RNA Isolation Kit, Gentra Systems Inc., Minneapolis, MN.
- Qiagen RNeasy Kit, Qiagen Inc., Valencia, CA.
- Invitrogen Inc., Carlsbad, CA.
- Applied BioSystems Inc., Foster City, CA.
- DNA Star Inc., Madison, WI.
- Biosearch Technologies Inc., Novato, CA.
- Bio-Rad Laboratories, Hercules, CA.
- Cepheid Inc., Sunnyvale, CA.

References

- Abdy MJ, Howerth EE, Stallknecht DE: 1999, Experimental infection of calves with epizootic hemorrhagic disease virus. *Am J Vet Res* 60:621-626.
- Aradaib IE, Smith WL, Osburn BI, Cullor JS: 2003, A multiplex PCR for simultaneous detection and differentiation of North American serotypes of bluetongue and epizootic hemorrhagic disease viruses. *Comp Immunol Microbiol Infect Dis* 26:77-87.
- Balasuriya UB, Nadler SA, Wilson WC, et al.: 2008, The NS3 proteins of global strains of bluetongue virus evolve into regional topotypes through negative (purifying) selection. *Vet Microbiol* 126:91-100.
- Barnard BJ, Gerdes GH, Meiswinkel R: 1998, Some epidemiological and economic aspects of a bluetongue-like disease in cattle in South Africa—1995/96 and 1997. *Onderstepoort J Vet Res* 65:145-151.
- Beaton AR, Rodriguez J, Reddy YK, Roy P: 2002, The membrane trafficking protein calpactin forms a complex with bluetongue virus protein NS3 and mediates virus release. *Proc Natl Acad Sci U S A* 99:13154-13159.
- Dubay SA, deVos JC, Jr, Noon TH, Boe S: 2004, Epizootiology of hemorrhagic disease in mule deer in central Arizona. *J Wildl Dis* 40:119-124.
- Edgar RC: 2004, MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792-1797.
- Flacke GL, Yabsley MJ, Hanson BA, Stallknecht DE: 2004, Hemorrhagic disease in Kansas: enzootic stability meets epizootic disease. *J Wildl Dis* 40:288-293.
- House C, Shipman LD, Weybright G: 1998, Serological diagnosis of epizootic hemorrhagic disease in cattle in the USA with lesions suggestive of vesicular disease. *Ann N Y Acad Sci* 849:497-500.
- Jensen MJ, Cheney IW, Thompson LH, et al.: 1994, The smallest gene of the orbivirus, epizootic hemorrhagic disease, is expressed in virus-infected cells as two proteins and the expression differs from that of the cognate gene of bluetongue virus. *Virus Res* 32:353-364.
- MacLachlan NJ, Osburn BI: 2004, Epizootic haemorrhagic disease of deer. *In: Infectious diseases for livestock*, ed. Coetzer JAW, Tustin RC, pp. 1227-1230. Oxford University Press, New York, NY.
- Mecham JO: 1993, Detection of bluetongue virus from blood of infected sheep by use of an antigen-capture enzyme-linked immunosorbent assay after amplification of the virus in cell culture. *Am J Vet Res* 54:370-372.
- Mecham JO, Dean VC: 1988, Protein coding assignment for the genome of epizootic haemorrhagic disease virus. *J Gen Virol* 69:1255-1262.
- Mecham JO, Jochim MM: 2000, Development of an enzyme-linked immunosorbent assay for the detection of antibody to epizootic hemorrhagic disease of deer virus. *J Vet Diagn Invest* 12:142-145.
- Mecham JO, Stallknecht D, Wilson WC: 2003, The S7 gene and VP7 protein are highly conserved among temporally and geographically distinct American isolates of epizootic hemorrhagic disease virus. *Virus Res* 94:129-133.
- Mecham JO, Wilson WC: 2004, Antigen capture competitive enzyme-linked immunosorbent assays using baculovirus-expressed antigens for diagnosis of bluetongue virus and epizootic hemorrhagic disease virus. *J Clin Microbiol* 42:518-523.
- Mertens PP, Pedley S, Cowley J, et al.: 1989, Analysis of the roles of bluetongue virus outer capsid proteins VP2 and VP5 in determination of virus serotype. *Virology* 170:561-565.

18. Murphy MD, Hanson BA, Howerth EW, Stallknecht DE: 2006, Molecular characterization of epizootic hemorrhagic disease virus serotype 1 associated with a 1999 epizootic in white-tailed deer in the eastern United States. *J Wildl Dis* 42:616–624.
19. Murphy MD, Howerth EW, MacLachlan NJ, Stallknecht DE: 2005, Genetic variation among epizootic hemorrhagic disease viruses in the southeastern United States: 1978–2001. *Infect Genet Evol* 5:157–165.
20. Ronquist F, Huelsenbeck JP: 2003, MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
21. Stallknecht DE, Howerth EW, Gaydos JK: 2002, Hemorrhagic disease in white-tailed deer: our current understanding of risk. *Trans North Am Wildl Nat Res Conf* 67:75–86.
22. Thompson JD, Gibson TJ, Plewniak F, et al.: 1997, The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882.
23. Thompson LH, Mecham JO, Holbrook FR: 1988, Isolation and characterization of epizootic hemorrhagic disease virus from sheep and cattle in Colorado. *Am J Vet Res* 49:1050–1052.
24. van Dijk AA, Huisman H: 1988, In vitro transcription and translation of bluetongue virus mRNA. *J Gen Virol* 69:573–581.
25. Verwoerd DW, Erasmus BJ: 2004, Bluetongue. *In: Infectious diseases of livestock*, ed. Coetzer JAW, Tustin RC, pp. 1021–1220. Oxford University Press, New York, NY.
26. Wilson WC: 1990, Development and optimization of a hybridization assay for epizootic hemorrhagic disease viruses. *J Virol Methods* 30:173–181.
27. Wilson WC: 1991, Molecular comparison of VP3 from bluetongue and epizootic hemorrhagic disease viruses. *Virus Res* 21:225–236.
28. Wilson WC: 1994, Development of a nested-PCR test based on sequence analysis of epizootic hemorrhagic disease viruses non-structural protein 1 (NS1). *Virus Res* 31:357–365.
29. Wilson WC, Bernard KA, Israel BA, Mecham JO: 2007, Bluetongue virus serotype 17 sequence variation associated with neutralization. *DNA Sequence* 19:237–240.
30. Wilson WC, Letchworth GJ, Jiménez C, et al.: 2009, Field evaluation of a multiplex real-time reverse transcription polymerase chain reaction assay for detection of Vesicular stomatitis virus. *J Vet Diagn Invest* 21. In press.
31. Wilson WC, Ma HC, Venter EH, et al.: 2000, Phylogenetic relationships of bluetongue viruses based on gene S7. *Virus Res* 67:141–151 [Erratum 173:201–202].
32. Wilson WC, Stallknecht DE, Mecham JO: 2004, Field-deployable real-time PCR detection of bluetongue and epizootic hemorrhagic disease viral RNA. *Vet Ital* 40:587–593.

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Comparative efficacy of conventional and TaqMan polymerase chain reaction assays in the detection of capripoxviruses from clinical samples

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Abstract. Sheeppox and goatpox are economically important viral diseases of sheep and goats, respectively. Both diseases are reportable to the World Organization for Animal Health. To implement a control and eradication program for these diseases, a rapid and user-friendly diagnostic tool is imperative for screening. Therefore, in the present study, TaqMan quantitative polymerase chain reaction (qPCR) and conventional PCR assays targeting the *DNA polymerase (DNA pol)* gene were developed for the detection of *Capripoxvirus* DNA from clinical specimens of sheep and goats. The 2 assays used different primer sets. Conventional PCR yielded a specific product of 134 bp, whereas qPCR yielded a 180-bp product. The specificity of amplified *DNA pol* gene products was confirmed by their size and by sequence analysis. The 2 assays were specific for *Sheeppox virus* and *Goatpox virus*. However, in comparison to conventional PCR, the qPCR was more rapid, specific, and 100 times more sensitive, with a detection limit as low as 0.042 pg of purified DNA. The qPCR assay was more sensitive (84.05%) than conventional PCR (76.06%) when used on clinical samples ($n = 71$) from sheep and goats.

Key words: Capripoxviruses; detection; *DNA pol* gene; polymerase chain reaction.

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Small ruminants comprise a vital component of livestock farming and provide livelihood to millions in the farming sector. The world population of sheep and goats is approximately 2.1 billion.⁴ Sheeppox and goatpox (collectively known as capripox diseases) are the most important World Organization for Animal Health notifiable viral diseases that pose serious socioeconomic impact