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Development and Validation of an Ovine Progressive Pneumonia Virus Quantitative PCR▽

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Ovine progressive pneumonia virus (OPPV) infects at least one sheep in 81% of U.S. sheep flocks, as determined by serology, and can cause viral mastitis, arthritis, dyspnea, and cachexia. Diagnostic tests that quantify OPPV proviral load in peripheral blood leukocytes (PBL) provide an additional method for identification of infected sheep and may help to further understanding of the pathogenesis of OPPV-induced disease. In this study, we compared a new OPPV real-time quantitative PCR (qPCR) assay specific for the transmembrane region of the envelope gene (*tm*) with a competitive inhibition enzyme-linked immunosorbent assay (cELISA) using 396 PBL samples and sera from Idaho sheep. The OPPV qPCR had a positive concordance of 96.2% ± 2.3% and a negative concordance of 97.7% ± 2.5% compared to the cELISA, with a kappa value of 0.93, indicating excellent agreement between the two tests. In addition, the presence of *tm* in the three OPPV qPCR-positive and cELISA-negative sheep and in 15 sheep with different OPPV proviral loads was confirmed by cloning and sequencing. These data indicate that the OPPV qPCR may be used as a supplemental diagnostic tool for OPPV infection and for measurement of viral load in PBLs of infected sheep.

Ovine progressive pneumonia virus (OPPV) is also referred to as maedi-visna virus (MVV) or ovine lentivirus (OvLV) and is a member of the family Retroviridae. As with other lentiviruses, infection with OPPV is defined as successful integration of virus into the sheep genome, and this integrated virus is called provirus. OPPV provirus is found in cells of the macrophage and monocyte lineage without causing cellular ablation (13, 14). Specifically, OPPV provirus has been detected in many cell types, including lung type I and II pneumocytes, interstitial and alveolar macrophages, endothelial cells, fibroblasts, colostrum cells, cumulus cells, peripheral blood mononuclear cells, and peripheral blood leukocytes (PBL) (2, 4, 6, 8, 11, 17, 18, 27). Plasma viremias are thought to be low in OPPV-infected sheep (3); therefore, measuring OPPV provirus loads may be the best way to quantitate virus in vivo.

Determination of the severity of OPPV-induced clinical disease has been primarily based upon postmortem pathological assessment of lung tissue, since sheep with lung lesions typically manifest one or more of various clinical signs, such as dyspnea, mastitis, arthritis, cachexia, and central nervous system disorders, prior to euthanasia (5). Subsequent reanalysis of the data from an earlier pathogenesis study showed that the presence of lung lesions (mild, moderate, or severe) correlates significantly with the presence of provirus in alveolar macrophages using PCR specific for *ltr* and *pol* (Fisher's exact test, two-sided P value = 0.0003) (4). In addition, sheep with lung lesions had >10-fold more *pol* copies in their alveolar macrophages, determined by a quantitative PCR (qPCR), than sheep without lung lesions (27). Currently, it is unknown whether OPPV provirus load in PBL can be used as predictor of clinical disease progression. However, obtaining PBL from a live sheep is a less complicated procedure than obtaining alveolar macrophages, which requires medical oversight during alveolar lavage.

There are neither treatments nor vaccines for OPPV; therefore, separation or culling of serologically positive sheep has been the primary control method of OPPV. Many agar gel immunodiffusion tests and ELISAs utilize region- or country-specific virus strains and/or antigens (9), and many of these serologic diagnostic tests lack sensitivity (resulting in elevated numbers of false negatives) when applied to flocks from different countries or regions. One reason for this decrease in sensitivity is the amino acid differences of the viral antigens between regions or countries. Many of the more sensitive serologically based ELISAs utilize synthetic peptides based on the transmembrane region of the envelope gene (*tm*) in conjunction with *gag* or *pol* products (7, 20). In an effort to produce another sensitive diagnostic test for OPPV infection and to establish a clinical prognostic test for OPPV, primers and a probe were designed according to the nucleotide sequence of the conserved *tm*, and a new real-time qPCR test for detection of OPPV provirus in PBL was developed. Cloning and sequencing of *tm* from representative sheep validated this new qPCR. In addition, this qPCR was evaluated for concordance against a previously validated cELISA, which utilizes a monoclonal antibody to the surface envelope glycoprotein (16, 23).

MATERIALS AND METHODS

Animals. Serum and PBL were isolated from 10 ml whole blood of 405 sheep of the U.S. Sheep Experiment Station, Dubois, ID, flock using previously de-

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scribed methods (18). There were 396 sheep in the final data set, comprising 135 Rambouillet, 129 Columbia, and 132 Polypay breed sheep, and 108 3-year-old, 101 4-year-old, 106 5-year-old, and 81 6-year-old sheep.

Serology diagnosis. The caprine arthritis-encephalitis virus (CAEV) cELISA (VMRD, Inc., Pullman, WA) was used to detect the presence of anti-OPPV antibodies in sheep serum following the manufacturer's directions and using negative control sheep sera. Validation of this CAEV cELISA for use in sheep was reported previously (16).

DNA isolation and OPPV qPCR. DNA was isolated from PBL following the manufacturer's directions for 10 million cells using Puregene technology (Gentra Systems Inc., Minneapolis, MN). The concentration and purity of DNA were determined spectrophotometrically at 260 and 280 nm. Primers and a TaqMan probe specific for *tm* in OPPV were designed using Beacon Designer (Premier Biosoft version 2.1) based upon a *tm* consensus sequence derived from plasmid *env* clones LMH15-6 pCR2.1, LMH16-3 pCR2.1, LMH17-5 pCR2.1, and LMH19-5 pCR2.1 from 4/10 OPPV-positive ewes (17). The *tm* TaqMan antisense probe was made at Integrated DNA Technologies, Coralville, IA: 5′-5′-hexachlorofluorescein-AGC AAC ACC GAG ACC AGC TCC TGC-3′ Black Hole Quencher-1. The OPPV qPCR primers TMENVCONf (5′-TCA TAG TGC TTG CTATCA TGG CTA-3′) and TMENVCONr (5′-CCG TCC TTG TGT AGG ATT GCT-3′) were ordered as desalted grade from Invitrogen Corporation, Carlsbad, CA. TMENVCONf binds to the nucleotide sequence at 1998 in GenBank accession U64439. In addition, primers and a TaqMan probe specific for exon 4 of the glyceraldehyde 3-phosphate dehydrogenase gene (*gapd*) were designed using Beacon Designer (Premier Biosoft version 2.1) and GenBank sequence AF272837. A *gapd* plasmid was constructed by PCR amplification of cDNA using the previously reported primers GAPDH 171 and GAPDH 172 and amplification conditions (15) followed by cloning into TOPO pCR2.1. The presence of the *gapd* sequence within the plasmid gapdh27Oct57 pCR2.1 was confirmed by Big Dye terminator sequencing (Applied Biosystems Inc., Foster City, CA). A *gapd* TaqMan antisense probe was made at Integrated DNA Technologies: 5′-5FAM (carboxyfluorescein)-CCG TTC TCT GCC TTG ACT TGT GCC G-3′ Black Hole Quencher-1. The primers gaprtf (5′-ATG TTC CAG TAT GAT TCC ACC CAT-3′) and gaprtr (5′-GCC TTT CCA TTG ATG ACG AGC-3′) were ordered as desalted grade from Invitrogen Corporation. TaqMan universal PCR 2X Master Mix (Applied Biosystems), 300 nM (final concentration) of either *tm* or *gapd* primers, 250 nM_f TaqMan *tm* or *gapd* probe, and up to 1 μg DNA isolated from peripheral blood mononuclear cells were used in a 50-μl total volume in real-time PCR. Amplification conditions for I-cycler IQ (Bio-Rad Laboratories, Hercules, CA) were 95°C for 10 min, 60 cycles of 95°C for 15 s, and 55°C for 60 s, and 4°C indefinitely. Triplicate reactions of control plasmid clone LMH17-5 pCR2.1 and gapdh27Oct57 pCR2.1 in separate reactions ranging from 10^0 to 10^7 copies were used to generate standard curves. Triplicate reactions of a negative animal control used all components above and DNA from PBL of a cELISA- and immunoprecipitation-seronegative ewe. Triplicate reactions of a negative reagent control used all components above, except that sterile water was substituted for DNA. Triplicate reactions of sample unknowns used all components above and up to 1 μg of DNA from each sheep.

Analysis of OPPV qPCR. A standard curve was constructed by plotting logarithmic copy number of the LMH17-5 pCR2.1 or gapdh27Oct57 pCR2.1 plasmid versus threshold cycle using a PCR baseline subtracted curve fit (Bio-Rad I-cycler software). Copy numbers of the unknown samples in the experiment were determined using the mean threshold cycle value and the equation of the line generated in the standard curve. The PCR efficiency was also calculated, as follows: $[10^{(-1/\text{slope})}] - 1$. The Bio-Rad I-cycler software calculated the mean copy number and the standard error of the mean copy number. Graphs were constructed using Prism version 4.0b (GraphPad Software Inc.).

***tm* analysis.** The OPPV qPCR primers TMENVCONf and TMENVCONr were utilized to PCR amplify the 90-bp *tm* fragment. This PCR amplicon was cloned into pCR2.1 using TOPO TA methods and sequenced using M13 forward and reverse primers by previously published methods (17). DNAStar Editseq and MegAlign software programs (Lasergene, Inc.) were used to align *tm* sequences to the consensus sequence representing the North American OPPV strains (EF207570 to EF207584).

RESULTS

Partial nucleotide sequences of the *tm* of several North American OvLVs (GenBank accession numbers EF207570 to EF207584) were utilized for the development of the *tm*-specific primers and probe for OPPV qPCR. The 5′ ends of the forward *tm* primer, antisense *tm* probe, and reverse *tm* primer bind to nucleotide positions 1998, 2034, and 2068, respectively, in *env* of GenBank accession number U64439. In addition, to control for the presence of DNA in an unknown sample, a plasmid standard, primers, and probe corresponding to the ovine glyceraldehyde 3-phosphate dehydrogenase gene (*gapd*) (GenBank accession number AF272837) were created. The 5′ ends of the forward *gapd* primer, antisense *gapd* probe, and reverse *gapd* primer bind to nucleotide positions 127, 162, and 189, respectively, in GenBank accession number AF272837, and these primers partially amplify exon 4 in *gapd*. To test whether the both the *gapd* and *tm* probes and primers can be used in a multiplex reaction, samples were run using both the *gapd* and *tm* primers and probes and run in parallel with the same samples using only the *tm* primers and probe. Unfortunately, there were $\sim 10^2$-fold fewer *env* copies/μg DNA in the multiplex reaction than in the single reaction. Therefore, since there were interaction problems with *gapd* and *tm* primers and probes, samples with the *gapd* primers and probe were run separately from samples with the *tm* primers and probe.

Proviral loads were detected by the OPPV qPCR in samples from 257 out of 396 naturally OPPV-exposed sheep and ranged from 1 copy to 62,700 copies of *env* per 1 μg DNA in PBL (Fig. 1A). In addition, *gapd* copy number ranged between 11,700 and 133,000 copies per 1 μg DNA in PBL with and without detectable *env* copies (data not shown). In order to assess the OPPV qPCR as an OPPV diagnostic test, OPPV qPCR test results were compared to results obtained with the previously validated cELISA (16). qPCR testing of 396 sheep of three breeds and four ages resulted in 96.2% positive concordance and 97.7% negative concordance (Table 1). There were 10 cELISA-positive and qPCR-negative samples and 3 cELISA-negative and qPCR-positive samples. The kappa statistic was 0.93, which indicates excellent agreement between the cELISA and OPPV qPCR (22).

gapd was detected in all 10 cELISA-positive and qPCR-negative samples; therefore, a lack of DNA was not the cause of failure for these 10 samples in the OPPV qPCR. To confirm the presence of *tm* in the three OPPV qPCR-positive and cELISA-negative animals, *tm* was amplified from genomic DNA of PBL using the qPCR primers, cloned, and sequenced. The *tm* from PBL of these three sheep showed 94.4%, 100%, and 100% nucleotide identity to the OPPV *tm* consensus sequence obtained from aligning GenBank accession numbers EF207570 to EF207584. In addition, to validate the presence of *tm* in sheep with various OPPV qPCR loads, the *tm* was cloned and sequenced from genomic DNA isolated from PBL of three sheep from five OPPV qPCR load categories. Load categories were determined based upon $\log_{10}$ amounts such that category 1 equals qPCR loads of 1 to 10 copies/μg DNA, and categories 2, 3, 4, and 5 equal qPCR loads of 11 to 100, 101 to 1,000, 1001 to 10,000, and 10,001 to 100,000 copies/μg DNA, respectively. The nucleotide sequence from these 15 sheep was confirmed as *tm* using sequence alignment software and ranged from 98.9 to 100% identical to the OPPV *tm* consensus sequence obtained from aligning GenBank accession numbers EF207570 to EF207584.

To evaluate whether age or breed affected the positive and negative concordance of the OPPV qPCR test with the cELISA, the percentage of sheep that were positive for

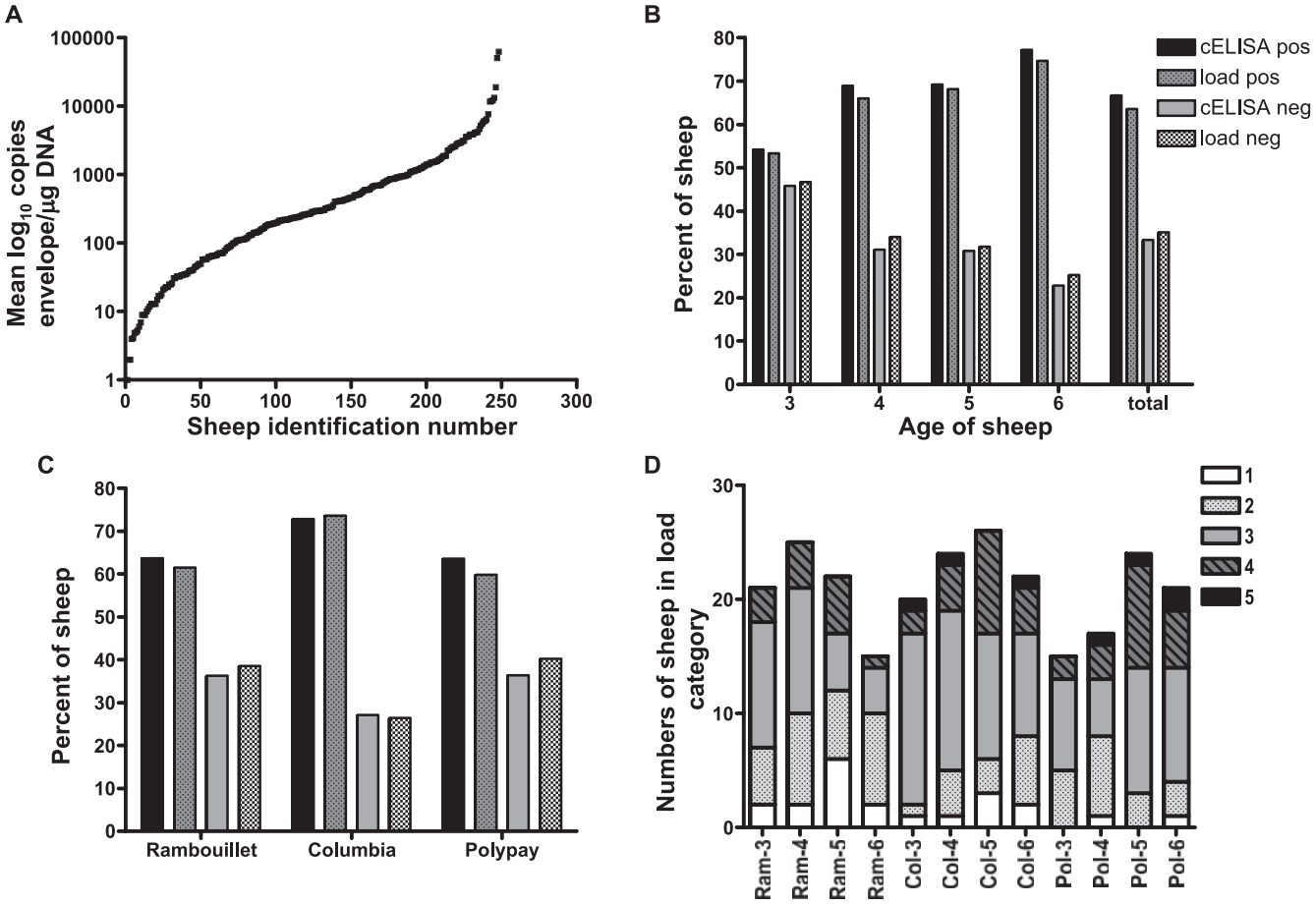

FIG. 1. (A) Mean $\log_{10}$ envelope loads plotted from least to greatest for the 257 sheep that had loads detectable by the OPPV qPCR assay. (B and C) cELISA and load status plotted against age (B) and breed (C). The key in panel B applies to panel C as well. (D) Numbers of sheep in each load category plotted against breed and age (e.g., Ram-3 indicates Rambouillet, 3 years of age).

cELISA, positive for load, negative for cELISA, and negative for load was plotted as a function of age (Fig. 1B) or breed (Fig. 1C). These results showed that OPPV qPCR results positively corresponded to cELISA results regardless of age or breed. In addition, in order to determine whether there were differences in the number of sheep in each OPPV qPCR load category with respect to age and breed, the numbers of sheep in the five load categories were plotted against breed and age (Fig. 1D). These data show that the seven sheep in load category 5 were either Columbia or Polypay and not Rambouillet. In fact, Rambouillet sheep had the highest number of sheep in load categories 1 and 2 with respect to age compared to Columbia and Polypay sheep. These results suggest that the OPPV qPCR test may serve to determine whether specific breeds are more resistant or susceptible to OPPV.

One question encountered in the development of a new diagnostic test is whether the test is reproducible. To check the reproducibility of the OPPV qPCR, both interassay and intra-assay experiments were conducted. Six separate qPCR assays (interassay) were conducted on 20 sheep in various load categories (Table 2). In terms of intra-assay variation, the same sheep samples that were analyzed in the interassay experiment

TABLE 1. Concordance of the OPPV qPCR to cELISA for detection of OPPV infection in sheep[a]

OPPV qPCR result	No. of samples with cELISA result	
	Positive[b]	Negative[c]
Positive	254	3
Negative	10	129
Total (concordance [CI[d]])	264 (254/264 [96.2 ± 2.3%])	132 (129/132 [97.7 ± 2.5%])

[a] The kappa value is 0.93, indicating excellent agreement.
[b] cELISA values of >20.9% inhibition.
[c] cELISA values of ≤20.9% inhibition.
[d] CI, 95% confidence interval.

TABLE 2. Interassay and intra-assay results using the OPPV qPCR assay

Sheep	Interassay		Intra-assay	
	No. of *env* provirus copies[a]	CV (%)	No. of *env* provirus copies[a]	CV (%)
1	5,473 ± 508	9	3,960 ± 416	11
2	4,825 ± 538	11	3,615 ± 376	10
3	3,691 ± 533	14	2,927 ± 148	5
4	3,272 ± 316	10	2,902 ± 112	4
5	1,921 ± 259	13	1,673 ± 193	12
6	1,654 ± 274	17	1,170 ± 130	11
7	1,189 ± 111	9	939 ± 133	14
8	902 ± 199	22	713 ± 103	14
9	562 ± 75	13	450 ± 34	8
10	488 ± 121	25	386 ± 60	15
11	344 ± 72	21	193 ± 18	9
12	291 ± 56	19	196 ± 23	11
13	240 ± 66	28	218 ± 51	23
14	191 ± 39	20	106 ± 26	25
15	187 ± 42	22	112 ± 18	16
16	56 ± 21	37	32 ± 8	25
17	47 ± 20	42	27 ± 9	33
18	46 ± 20	42	26 ± 6	22
19	39 ± 14	36	23 ± 5	20
20	19 ± 11	58	13 ± 5	35

[a] Mean ± standard deviation for six replicates.

were utilized and were tested six times in a single qPCR run (Table 2). Lower coefficients of variation were observed in the intra-assay than in the interassay experiments. Overall, sheep with provirus loads greater than 100 copies/μg DNA in the intra-assay had lower coefficients of variation (CV; range, 4 to 25%) than sheep with provirus loads less than 100 copies/μg DNA (range, 20 to 35%).

Since OPPV infects monocytes/macrophages, and monocytes typically make up 1 to 6% of PBL, 1 μg was chosen as the starting quantity in the OPPV qPCR in order to maximize the chance of detecting integrated OPPV in monocytes. However, 1 μg of DNA per PCR is considered excessive and may cause PCR inhibition. To check whether the use of high quantities of DNA affects the measurement of copy number, four 10-fold dilutions of DNA starting at 1 μg from three sheep of each load category were run in triplicate in the OPPV qPCR. Figure 2 shows the amount of DNA run in the qPCR plotted against the mean $\log_{10}$ copy number of *env* for each animal. The mean $\log_{10}$ copy number of *env* was calculated by multiplying the copy number of envelope by the dilution factor. These data showed that loads from animals in load categories 1 and 2 are undetectable when less than 1 μg of DNA is used in the OPPV qPCR.

DISCUSSION

Two explanations for the sigmoid dose response curve of qPCR loads are that flock mates are infected with different amounts of virus or are at different stages of infection. Most transmission of OPPV is believed to occur horizontally; however, the major reservoir hosts have not been fully elucidated (1, 10, 18, 19, 26). An animal with high OPPV loads (category 5) in its PBL has the potential to transmit more virus. In this data set, only 7 out of 257 qPCR-positive animals were in load category 5 or maintained 10,001 to 100,000 copies/μg DNA, and they were either Columbia or Polypay sheep. Currently, infectious disease research hypothesizes that 80% of the infectious disease transmission occurs from the highly infected (20%) (12, 21). It is possible that these seven Columbia and Polypay sheep were more efficient in transmitting OPPV horizontally, since they had the highest qPCR proviral loads. The determination of OPPV load by qPCR in concert with characterization of the viral strain or strains should further the understanding of transmission efficiency of virus from sources such as colostrum and milk.

The finding that three OPPV qPCR-positive and cELISA-negative sheep were confirmed load positive by cloning and sequencing *tm* indicates that in some animals, the detection of integrated *env* may precede or be more sensitive than the measurement of antibody response by cELISA to the surface envelope glycoprotein. In this respect, the OPPV qPCR may serve as a confirmatory or supplemental test for determining the infection status of an animal. More importantly, verification of 15 load-positive animals with loads ranging from 1 to 10^4+ copies/μg DNA by sequencing *tm* from these animals validates the use of this new OPPV qPCR assay as an OPPV diagnostic test.

In addition, the intra-assay and interassay variability revealed that the OPPV qPCR reproducibility was much higher in animals with more than 100 copies/μg DNA. However, to ensure detection of less than 100 copies/μg DNA in some sheep, 1 μg should be used in the OPPV qPCR. With this new OPPV qPCR, interassay and intra-assay reproducibility may have to be sacrificed for detection of provirus in the OPPV qPCR, especially in sheep with less than 100 copies/μg DNA. By including a separate measurement of *gapd* in only those samples where *env* copies were undetectable, diagnostic testing costs can be lowered, and the presence of DNA in apparently false-negative *env* samples can be confirmed.

Interestingly, the *tm* primers and probe were not able to amplify CAEV provirus from goat synovium membrane cells infected with CAEV-63 (data not shown). The partial nucleo-

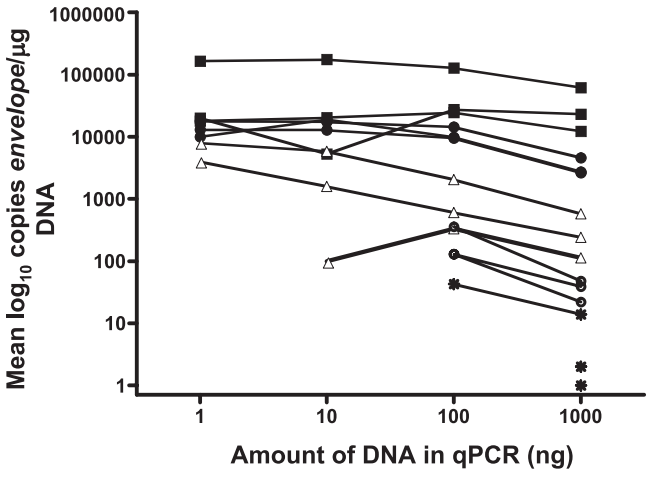

FIG. 2. Mean $\log_{10}$ copies of *env* plotted against the amount of DNA used in the qPCR for three sheep in load categories 1 (stars), 2 (open circles), 3 (open triangles), 4 (filled circles), and 5 (filled squares).

tide sequences of *tm* at nucleotide positions 1985 through 2108 in *env* from GenBank accession U64439 are 88.6 to 95.9% identical between Dubois OPPV strains (EF207570 to EF207584) and 1514-LV1-1KS1 (M60609 and M37977), EV1 (S51392), KM1071 (U51910), 1772 (L06906), and South African MVV (M31646), whereas only 71.5 to 75.6% nucleotide identity was observed between the Dubois OPPV strains and CAEV-63 (M60855). The fact that the OPPV qPCR does not amplify CAEV provirus suggests that the OPPV qPCR reported here might be able to differentiate CAEV from OPPV infection, at least with the strains tested in this study. This is important, since there are phylogenetic reports indicating that CAEV infects sheep and MVV infects goats under natural field conditions (24, 25). In the future, goats infected with natural field strains of CAEV will be evaluated using the OPPV qPCR, and if the OPPV qPCR fails for the goats, a CAEV-specific qPCR will be developed.

In conclusion, since the positive and negative concordances of the OPPV qPCR and cELISA are 96.2 and 97.7%, respectively, and the qPCR and cELISA positively correlate with respect to age and to breed, this OPPV qPCR provides an additional method for detecting the presence of OvLV infection by the OPPV strains in this study. Additional evaluation of field samples from other locations will aid in assessing the breadth of the sensitivity and specificity of this OPPV qPCR in sheep infected with different OPPV or MVV strains. The use of this qPCR should help to further our understanding of the pathogenesis of OPPV infection.

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REFERENCES

1. **Alvarez, V., J. Arranz, M. Daltabuit, I. Leginagoikoa, R. A. Juste, B. Amorena, D. de Andres, L. L. Lujan, J. J. Badiola, and E. Barriatua.** 2005. Relative contribution of colostrum from maedi-visna virus (MVV) infected ewes to MVV-seroprevalence in lambs. Res. Vet. Sci. **78:**237–243.
2. **Alvarez, V., M. Daltabuit-Test, J. Arranz, I. Leginagoikoa, R. A. Juste, B. Amorena, D. de Andres, L. L. Lujan, J. J. Badiola, and E. Barriatua.** 2006. PCR detection of colostrum-associated Maedi-Visna virus (MVV) infection and relationship with ELISA-antibody status in lambs. Res. Vet. Sci. **80:**226–234.
3. **Brahic, M., L. Stowring, P. Ventura, and A. T. Haase.** 1981. Gene expression in visna virus infection in sheep. Nature **292:**240–242.
4. **Brodie, S. J., K. A. Marcom, L. D. Pearson, B. C. Anderson, A. de la Concha-Bermejillo, J. A. Ellis, and J. C. DeMartini.** 1992. Effects of virus load in the pathogenesis of lentivirus-induced lymphoid interstitial pneumonia. J. Infect. Dis. **166:**531–541.
5. **Brodie, S. J., L. D. Pearson, M. C. Zink, H. M. Bickle, B. C. Anderson, K. A. Marcom, and J. C. DeMartini.** 1995. Ovine lentivirus expression and disease virus replication, but not entry, is restricted to macrophages of specific tissues. Am. J. Pathol. **146:**250–263.
6. **Carrozza, M. L., M. Mazzei, P. Bandecchi, M. Arispici, and F. Tolari.** 2003. In situ PCR-associated immunohistochemistry identifies cell types harbouring the Maedi-Visna virus genome in tissue sections of sheep infected naturally. J. Virol. Methods **107:**121–127.
7. **Celer, V., Jr, and V. Celer.** 2001. Detection of antibodies to ovine lentivirus using recombinant capsid and transmembrane proteins. J Vet. Med. B **48:** 89–95.
8. **Cortez Romero, C., F. Fieni, C. Roux, P. Russo, J. M. Guibert, F. Guiguen, Y. Chebloune, M. Pepin, and J. L. Pellerin.** 2006. Detection of ovine lentivirus in the cumulus cells, but not in the oocytes or follicular fluid, of naturally infected sheep. Theriogenology **66:**1131–1139.
9. **de Andrés, D., D. Klein, N. J. Watt, E. Berriatua, S. Torsteinsdottir, B. A. Blacklaws, and G. D. Harkiss.** 2005. Diagnostic tests for small ruminant lentiviruses. Vet. Microbiol. **107:**49–62.
10. **De Boer, G. F., C. Terpstra, D. J. Houwers, and J. Hendriks.** 1979. Studies in epidemiology of maedi-visna in sheep. Res. Vet. Sci. **26:**202–208.
11. **Eltahir, Y. M., C. I. Dovas, M. Papanastassopoulou, M. Koumbati, N. Giandinis, S. Verghese-Nikolakaki, and G. Koptopoulos.** 2006. Development of a semi-nested PCR using degenerate primers for generic detection of small ruminant lentivirus proviral DNA. J. Virol. Methods **135:**240–246.
12. **Galvani, A. P., and R. M. May.** 2005. Epidemiology: dimensions of superspreading. Nature **438:**293–295.
13. **Gendelman, H. E., O. Narayan, S. Kennedy-Stoskopf, P. G. Kennedy, Z. Ghotbi, J. E. Clements, J. Stanley, and G. Pezeshkpour.** 1986. Tropism of sheep lentiviruses for monocytes: susceptibility to infection and virus gene expression increase during maturation of monocytes to macrophages. J. Virol. **58:**67–74.
14. **Gorrell, M. D., M. R. Brandon, D. Sheffer, R. J. Adams, and O. Narayan.** 1992. Ovine lentivirus is macrophage tropic and does not replicate productively in T lymphocytes. J. Virol. **66:**2679–2688.
15. **Herrmann, L. M., W. C. Davis, D. P. Knowles, K. J. Wardrop, M.-S. Sy, P. Gambetti, and K. I. O'Rourke.** 2001. Cellular prion protein is expressed on peripheral blood mononuclear cells but not platelets of normal and scrapie infected sheep. Haematologica **86:**146–153.
16. **Herrmann, L. M., W. P. Cheevers, T. C. McGuire, D. S. Adams, M. M. Hutton, W. G. Gavin, and D. P. Knowles.** 2003. Detection of serum antibodies to ovine progressive pneumonia virus in sheep by using a caprine arthritis-encephalitis virus competitive-inhibition enzyme-linked immunosorbent assay. Clin. Diagn. Lab. Immunol. **10:**862–865.
17. **Herrmann, L. M., I. Hötzel, W. P. Cheevers, K. Pretty On Top, G. S. Lewis, and D. P. Knowles.** 2004. Seven new ovine progressive pneumonia virus (OPPV) field isolates from Dubois Idaho sheep comprise part of OPPV clade II based on surface envelope glycoprotein (SU) sequences. Virus Res. **102:**215–220.
18. **Herrmann-Hoesing, L. M., G. H. Palmer, D. P. Knowles.** 2007. Evidence of proviral clearance following postpartum transmission of an ovine lentivirus. Virology **362:**226–234.
19. **Houwers, D. J., C. D. Konig, G. F. De Boer, and J. Schaake, Jr.** 1983. Maedi-visna control in sheep. I. Artificial rearing of colostrum-deprived lambs. Vet. Microbiol. **8:**179–185.
20. **Kwang, J., J. Keen, R. C. Cutlip, and E. T. Littledike.** 1993. Evaluation of an ELISA for detection of ovine progressive pneumonia antibodies using a recombinant transmembrane envelope protein. J. Vet. Diagn. Investig. **5:**189–193.
21. **Lloyd-Smith, J. L., S. J. Schreiber, P. E. Kopp, W. M. Getz.** 2005. Superspreading and the effect of individual variation on disease emergence. Nature **438:**355–359.
22. **Martin, S. W., A. H. Meek, and P. Willeberg.** 1987. Measurement of disease frequency and production, p. 58–59. *In* S. Martin et al. (ed.), Veterinary epidemiology: principles and methods. Iowa State University Press, Ames, IA.
23. **Özyörük, F., W. P. Cheevers, G. A. Hullinger, T. C. McGuire, M. Hutton, and D. P. Knowles.** 2001. Monoclonal antibodies to conformational epitopes of the surface glycoprotein of caprine arthritis-encephalitis virus: potential application to competitive-inhibition enzyme-linked immunosorbent assay for detecting antibodies in goat sera. Clin. Diagn. Lab. Immunol. **8:**44–51.
24. **Pisoni, G., A. Quasso, and P. Moroni.** 2005. Phylogenetic analysis of small-ruminant lentivirus subtype B1 in mixed flocks: Evidence for natural transmission from goats to sheep. Virology **339:**147–152.
25. **Shah, C., J. B. Huder, J. Boni, M. Schonmann, J. Muhlherr, H. Lutz, and J. Schupbach.** 2004. Direct evidence for natural transmission of small-ruminant lentiviruses of subtype A4 from goats to sheep and vice versa. J. Virol. **78:**7518–7522.
26. **Sigurdsson, B., P. A. Palsson, and A. Tryggvaddottir.** 1953. Transmission experiments with maedi. J. Infect. Dis. **93:**166–175.
27. **Zhang, Z., N. J. Watt, J. Hopkins, G. Harkiss, and C. J. Woodall.** 2000. Quantitative analysis of maedi-visna virus DNA load in peripheral blood monocytes and alveolar macrophages. J. Virol. Methods **86:**13–20.