

Supplemental Table 1. Primer and probe sequences for viral PCR assays designed in-house.

| Oligonucleotide name | Target | Sequence 5'–3' | Final concentration, μ M |
|-----------------------|-----------------|----------------------------------|------------------------------|
| BoAstV_ORF2_191119-F | ORF2 | GTTTGAATGCCTGCGTAGC | 0.50 |
| BoAstV_ORF2_191119-R | ORF2 | CCTGTACCCTCGATCCTACT | 0.50 |
| BoAstV_ORF2_191119-Pr | ORF2 | FAM/AARTCTATGAGGGCGTGCGYGG | 0.25 |
| BoAstV_CH15-F | ORF2 | CTTAGAGGCCACGCAGAAG | 0.50 |
| BoAstV_CH15-R | ORF2 | CCTGTACCCTCGATCCTACTC | 0.50 |
| BoAstV_CH15-Pr | ORF2 | HEX/CGGTTGCCGTAGTGAAATCAATACAACC | 0.25 |
| MCF_gB2_210901_F | <i>gB2</i> gene | ATCGTGCTGAACCTGAC | 0.50 |
| MCF_gB2_210901_R | <i>gB2</i> gene | AGTTGTACTCCCTGAACAT | 0.50 |
| MCF_gB2_210901_Pr | <i>gB2</i> gene | FAM/AAGCGCATGAGCACGGT | 0.25 |
| BoAHV1_140118-F | <i>gB</i> gene | GCACACGACGGACGATG | 0.50 |
| BoAHV1_140118-R | <i>gB</i> gene | TCCACTTCTTCCACGATGC | 0.50 |
| BoAHV1_140118-Pr | <i>gB</i> gene | FAM/CACGGGCACCTCTGTGAAC | 0.25 |
| BPIV3_P_140118-F | <i>P</i> gene | AGGACACAGAAGAGAGCAC | 0.50 |
| BPIV3_P_140118-R | <i>P</i> gene | AGGACATTCCGCCACACA | 0.50 |
| BPIV3_P_140118-Pr | <i>P</i> gene | HEX/TCTTGGTGTAATCCAATCTGCAGCA | 0.25 |
| BRSV_N_140118-F | <i>N</i> gene | TCAACTAGAGGAGGTAGTAGGGT | 0.50 |
| BRSV_N_140118-R | <i>N</i> gene | GCTGGCATGACCAAGCATAA | 0.50 |
| BRSV_N_140118-Pr | <i>N</i> gene | TexRd/TGCATATGGAGCAGGTCAAGTGATGT | 0.25 |
| Entero_31_For | 5'-UTR | ATGCGGCTAATCCCAACCT | 0.20 |
| Entero_31_Rev | 5'-UTR | CGTTACGACAGGCCAATCACT | 0.20 |
| Entero_LNA_probe | 5'-UTR | Cy5/CA+G+GTGGTCA+C+AAAC | 0.06 |

BoAHV1 = bovine alphaherpesvirus 1; BoAstV = bovine astrovirus; BPIV3 = bovine parainfluenza virus 3; BRSV = bovine respiratory syncytial virus; Entero = enterovirus (internal control PCR); MCF = malignant catarrhal fever (ovine gammaherpesvirus 2).

Supplemental Table 2. Cycling parameters for viral PCR assays.

| Step | Temperature, °C | Hold, min | Ramp, °C/s | Cycles, X |
|-----------------------|-----------------|-----------|------------|-----------|
| Reverse transcription | 45 | 10:00 | 4.4 | 1 |
| Activation | 95 | 10:00 | 4.4 | 1 |
| Denaturation | 94 | 0:05 | 2.2 | 45 |
| Annealing/extension | 60 | 1:00 | 2.2 | 45 |
| Cool | 40 | 10:00 | 2.2 | 1 |

Supplemental Table 3. Primers and probe for the *Listeria monocytogenes* qPCR assay.

| Name | Sequence 5'–3' |
|----------------|----------------------------------|
| Forward primer | CGGCGCACCTAACCAAGTAA |
| Reverse primer | CAGTCTGGACAATCTCTTTGAATTTT |
| Probe (FAM) | TCAAGATGACTACAATGGTCCGAGTGTGAAAA |

Supplemental Table 4. Reaction conditions for the *Listeria monocytogenes* qPCR assay.

| Program | Cycles | Target temperature, °C | Time | Temperature transition, °C/s | Acquisition mode |
|----------|--------|------------------------|--------|------------------------------|------------------|
| Denature | 1 | 95 | 2 min | 4.8 | None |
| PCR | 45 | 95 | 5 sec | 4.8 | None |
| | | 60 | 30 sec | 2.5 | Single |
| Cool | 1 | 40 | 10 sec | 2.5 | None |

Supplemental Table 5. Data for all animals in the study.

| Group | Year | Age | Sex | BoAstV | RABV | BVDV | WNV | EEEV | OvGHV2 | BoAHV1 | Listeria | Chlam | BCoV | BAdV |
|-------|------|-------|-----|--------|------|------|-----|------|--------|--------|----------|-------|------|------|
| LE | 1990 | 12 mo | M | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1990 | 15 mo | F | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1994 | 12 mo | F | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1995 | 5 y | F | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| LE | 1995 | 16 mo | F | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| LE | 1996 | 12 mo | M | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1996 | 15 mo | F | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| LE | 1996 | Adult | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1996 | 6 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1997 | Adult | NS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2014 | 7 mo | M | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2016 | 2 y | M | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2016 | 12 mo | M | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| LE | 2017 | 7 y | F | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| LE | 2012 | 20 mo | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2009 | 8 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2003 | Adult | NA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2004 | 1.5 y | NA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2002 | Adult | NA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2002 | Adult | NA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2002 | Adult | NA | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2002 | Adult | NA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2002 | Adult | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2002 | Adult | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2002 | Adult | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Group | Year | Age | Sex | BoAstV | RABV | BVDV | WNV | EEEV | OvGHV2 | BoAHV1 | Listeria | Chlam | BCoV | BAdV |
|-------|------|-------|-----|--------|------|------|-----|------|--------|--------|----------|-------|------|------|
| LE | 2002 | Adult | NA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2001 | Adult | NA | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2001 | 4 mo | M | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| LE | 2001 | Adult | NA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2007 | 1 y | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2008 | 8 mo | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2009 | 7 mo | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2010 | Adult | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2011 | 2 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2013 | 10 mo | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2012 | 1 y | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2014 | 1 y | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2014 | 10 mo | M | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| LE | 2010 | 1 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2012 | 6 mo | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2015 | 8 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2017 | 5 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2019 | 4 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2019 | 3 y | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 2019 | 2 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1991 | 2 y | F | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| LE | 1992 | 2 y | F | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| LE | 1992 | 6 mo | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1993 | 6 mo | M | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1993 | Adult | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| LE | 1993 | 2 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1995 | 5 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Group | Year | Age | Sex | BoAstV | RABV | BVDV | WNV | EEEV | OvGHV2 | BoAHV1 | Listeria | Chlam | BCoV | BAdV |
|-------|------|-------|-----|--------|------|------|-----|------|--------|--------|----------|-------|------|------|
| LE | 1996 | Adult | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| LE | 1996 | 9 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1998 | Adult | F | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| LE | 1996 | 6 y | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| OE | 2012 | 2 y | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2019 | Adult | NA | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2018 | 9 mo | NA | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2018 | 1 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2017 | 5 y | NA | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2017 | 7 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2016 | 6 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2014 | 1 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2014 | 7 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2014 | 9 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2014 | 1 y | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2014 | 1 y | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2001 | 7 mo | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2001 | Adult | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2002 | Adult | NA | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2002 | Adult | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2002 | Adult | NA | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2004 | 6 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2013 | 6 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2012 | 9 mo | NA | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 1998 | 9 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2002 | 7 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2002 | Adult | NA | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |

| Group | Year | Age | Sex | BoAstV | RABV | BVDV | WNV | EEEV | OvGHV2 | BoAHV1 | Listeria | Chlam | BCoV | BAdV |
|-------|------|-------|-----|--------|------|------|-----|------|--------|--------|----------|-------|------|------|
| OE | 2003 | Adult | NA | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2004 | 1.5 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2008 | 5 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2009 | 10 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2010 | 1 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2011 | 2 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2012 | 8 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2008 | 4 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| OE | 2009 | 7 mo | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2009 | 11 mo | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2009 | 7 mo | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2009 | 5 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2009 | Adult | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2009 | 7 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2010 | 5 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2010 | Adult | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2010 | 7 mo | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2010 | 7 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2011 | 2 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 1999 | Adult | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2001 | Adult | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2001 | Adult | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2002 | Adult | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2002 | 6 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2002 | Adult | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2003 | 11 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2004 | 2 y | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |

| Group | Year | Age | Sex | BoAstV | RABV | BVDV | WNV | EEEV | OvGHV2 | BoAHV1 | Listeria | Chlam | BCoV | BAdV |
|-------|------|-------|-----|--------|------|------|-----|------|--------|--------|----------|-------|------|------|
| NE | 2004 | 1 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2005 | 6 mo | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2006 | 1 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2006 | 13 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2007 | 3 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2008 | 5 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2012 | 6 mo | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2012 | 1.5 y | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2013 | Adult | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2013 | 7 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2014 | 6 mo | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2014 | 1 y | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2014 | 10 mo | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2015 | 4 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2015 | Adult | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2015 | 2 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2016 | 7 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2016 | 3 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2017 | 1 y | M | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2017 | 4 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2019 | 2 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| NE | 2019 | 6 y | F | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |

BAdV = bovine adenovirus; BoAHV1 = bovine alphaherpesvirus 1; BoAstV = bovine astrovirus CH13/NeuroS1; BVDV = bovine viral diarrhea virus; Chlam = *Chlamydia* spp.; BCoV = bovine coronavirus; EEEV = eastern equine encephalitis virus; F = female; LE = lymphocytic encephalitis; Listeria = *Listeria monocytogenes*; M = male; OE = other forms of encephalitis; OvGHV2 = ovine

herpesvirus 2 (malignant catarrhal fever); NA = not available; ND = not tested; NE = no encephalitis (no brain disease); WNV = West Nile virus.