

1 **CROSS-CANADA DISEASE REPORT/**

2 **RAPPORT DES MALADIES DIAGNOSTIQUÉES AU CANADA**

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4 **QUEBEC**

5 **Detection of bovine lymphotropic herpesvirus DNA in tissues of a bovine aborted fetus**
6 **in Quebec**

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10 In late 2008, the Molecular Biology Diagnostic Laboratory (MBDL) of the Faculty of Veterinary
11 Medicine, University of Montreal initiated a research project to investigate the etiological agents
12 involved in cow abortion in Quebec, Canada. Several reverse-transcriptase polymerase chain reaction
13 (RT-PCR) and PCR-based assays were done on placenta and tissues of aborted fetuses that were
14 submitted to the MBDL for the detection of bovine pathogens that are known to be involved in abortion
15 such as bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis herpesvirus (IBR),
16 *Neospora caninum*, *Leptospira* spp. and *Coxiella burnetii* (1, 2). From the 26 submitted cases of bovine
17 abortion that were tested, none were positive for BVDV and *Leptospira* spp., whereas 3.8%, 11.5%, and
18 3.8% were positive for IBR, *Neospora caninum*, and *Coxiella burnetii*, respectively (Table 1).

19 In addition to the usual PCR assays, a pan-herpesvirus nested-PCR assay, which is able to detect
20 a broad spectrum of herpesvirus species, was conducted on placenta and pooled tissues of aborted
21 fetuses as previously described (3). Briefly, DNA was extracted from samples with the QIAamp DNA
22 mini kit (QIAGEN, Mississauga, Ontario) according to the manufacturer's tissue protocol. The PCR was
23 carried out in a Biometra T3 thermocycler with the QIAGEN Fast Cycling PCR kit according to the
24 manufacturer's specification with a set of 3 primers (DFA, ILK, and KG1). Amplification conditions

25 were: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 96°C for 5 s,
26 annealing at 46°C for 12 s, and extension at 68°C for 45 s. Reaction was ended with a final extension
27 step of 1 min at 72°C. Nested-PCR was then performed under the same conditions using 5 µL of the
28 first reaction and 2 other primers (TGV, IYG).

29 In regards to this pan-herpesvirus nested-PCR assay, excluding the IBR positive cases, one
30 submitted sample (FMV09-1125585) proved positive (Table 1). Interestingly, PCR fragment
31 sequencing and GenBank comparison by BLAST showed high homology with the DPOL viral gene of a
32 bovine lymphotropic herpesvirus (BLHV), which is a virus classified in the *Herpesviridae* family within
33 the subfamily *Gammaherpesvirinae* (4). Since the first PCR assay amplifies a rather small DNA
34 fragment [< 300 base pairs (bp)], and to further characterize the viral genome, the primers DFA and
35 IYG, encompassing a 483 nucleotides fragment of the DPOL gene, were used in a PCR assay. The
36 resulting amplified DNA was sequenced and compared with other herpesvirus DNA sequences using the
37 BioEdit software (BioEdit Sequence Alignment Editor version 7.0.5.2, Ibis Therapeutics; Carlsbad,
38 California, USA) and the CLUSTAL W alignment method. The FMV09-1125585 BLHV DNA
39 sequence (GenBank accession number HM152484) possesses a 100% nucleotides identity with another
40 GenBank reported BLHV sequence (GenBank accession number AF327830) (data not shown). To our
41 knowledge, this is the first time that BLHV has been found in the bovine species in Canada. BLHV has
42 been previously reported only in two countries; United States (US) in 1998 (4) and United Kingdoms
43 (UK) in 2006 (5). The prevalence of BLHV seems very high, the virus was identified by PCR in 91% of
44 tested adult animals ($n=101$) originating from Colorado, New York and New Jersey US States (4) and in
45 27% of the UK tested samples ($n=66$ tested samples) from cows experiencing non-responsive post-
46 partum metritis (NPPM) (6). Interestingly, the prevalence of BLHV in young animals seems also to be

47 very high, the virus having been identified by PCR in 38% of the tested 2 weeks old calves ($n=13$) (6).
48 Unfortunately, the BLHV transmission mode is unknown.

49 Since the first assay on a pool of tissues of the aborted fetus gave a negative result and to further
50 investigate the case, individual tissues were tested by nested-PCR and amplicons that were produced
51 were sequenced. Interestingly, brain and lymph nodes were BLHV positives and, to our knowledge, this
52 is the first time that BLHV was detected in tissues of a bovine aborted fetus. Noteworthy, BLHV has
53 been previously found by PCR within lymphoma cells, peripheral blood mononuclear cells (PBMC),
54 vaginal exudates and vaginal swabs (4-6). It is not surprising to have found BLHV DNA in brain and
55 lymph nodes tissues in an aborted fetus since herpesviruses are well known to possess a tropism for
56 neuronal cells and lymphocytes (7). Samples of spleen, liver, kidney, and heart were negative.

57 In regards to FMV09-1125585 clinical case, the submitted tissues were from a 2nd consecutive
58 abortion in the herd of 100 animals and the 5th one during a 12-month period. Placenta and pool of
59 tissues were also PCR-positive for *Neospora caninum* (one of the 3 cases presented in Table 1), which is
60 an important pathogen involved in cow abortion (2). Histopathological findings revealed a mild and
61 multifocal neutrophilic placentitis. In the liver of the aborted fetus, there was marked extramedullary
62 hematopoiesis. Small intrasinusoidal thrombi were found multifocally within the hepatic parenchyma
63 and were often associated with necrosis of a variable number of hepatocytes. There was no significant
64 lesion in the sections of brain, heart, lung, spleen and kidney examined. Unfortunately, lymph nodes
65 were not submitted for microscopic evaluation.

66 Even if others have reported the presence of BLHV with bovine leukemia virus (BLV) within
67 tumor cells and in samples originating from dairy cows experiencing NPPM, the involvement of BLHV
68 in the induction of any diseases is still uncertain (4-6). In addition, the BLV and BLHV status of the
69 dam is unknown and no histopathological lesion suggestive of a viral infection could be found in BLHV

70 nested-PCR positive tissues. Thus, it is very difficult to ascertain that BLHV has a role to play in the
71 cause of the abortion. Moreover, the presence of *Neospora caninum* was confirmed by PCR in the
72 BLHV positive case. Nonetheless, it is important to report the presence of BLHV in Canada because this
73 is the first step toward the country's improvement of bovine pathogen surveillance.

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75 **Acknowledgments**

76 This work was supported by the Natural Sciences and Engineering Research Council of Canada
77 (NSERC) discovery grant (to CAG). The authors are grateful to Dr. Paul Baillargeon (Pfizer Canada)
78 for the submission of bovine aborted fetuses tissue samples to the Diagnostic Service of the Faculté de
79 médecine vétérinaire, Université de Montréal and for covering the cost of the BVDV and IBR PCR
80 assays.

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