Cross-Canada Disease Report
Rapport des maladies diagnostiquées au Canada

Québec

First reported outbreak of Duck atadenovirus A tracheobronchitis in 3-week-old ducklings in Québec including whole genome sequence of the virus

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Duck atadenovirus A, also known as Duck adenovirus 1 (DAdV-1), is a member of the genus *Atadenovirus* (1). This virus is also responsible for the Egg Drop Syndrome (EDS) of laying hens (2). This syndrome is listed as an immediately notifiable disease in Canada, because it is considered as an exotic disease for which there are no control or eradication programs (3).

Although this virus has never been reported in commercial chicken production in Canada, DAdV-1 was identified in 2009 in 2 Muscovy duck farms in Ontario (4). An adenovirus was also isolated in goslings with respiratory clinical signs in Saskatchewan in 1992, but the adenovirus species was undetermined (5,6). Antibodies to DAdV-1 have been detected in numerous wild waterfowl in North America, up to 42% in sampled ducks, suggesting it is widespread on the continent and that these birds act as reservoirs (7). Overall, DAdV-1 seems weakly pathogenic for ducks and geese because most infections are asymptomatic, and the rare reports of clinical signs always imply very young (4 to 20 d of age) ducklings and goslings. When sick, these birds show gasping, dyspnea, coughing, and/or sneezing and mortality is moderate (2% to 7%) (4–6). DAdV-1 is substantially more pathogenic in chicken layers, in which it is associated with a severe drop in egg production that could last for 4 to 10 wk, and the laying of thin-shelled, soft-shelled, or shell-less eggs (2).

The first clinical case of DAdV-1 in a commercial duck farm in Québec is described herein, with whole genome sequencing of the virus.

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Case description

In June 2019, a duck producer called his veterinarian for mortality associated with possible cough in 3-week-old ducklings. This producer owned a multi-age broiler duck farm located in Québec, on one site which had several buildings. Sick animals were part of a group of 325 birds, housed on the 2nd floor of a building in which they were separated from another group of 2-week-old birds by a 16-inch plain barrier (same ventilation). This building also housed 2 lots of 10- and 11-week-old ducklings on the first floor. Except for the group of 3-week-old ducklings, no other group experienced clinical signs. Interestingly, only females (n = 25) were sick in the affected group and all other groups were composed of males only. It was the first time that this producer had bought females from his regular hatchery, to satisfy one of his clients who wanted this product.

Five 3-week-old dead female ducklings were submitted to the Laboratoire de Santé Animale de St-Hyacinthe. Gross examination revealed white soft plugs in their trachea and/or bronchi, obstructing their lumen (Figure 1). The tracheal mucosa was moderately congested. On histopathology, the tracheal epithelium was severely hyperplastic, with extensive loss of cilia and glands. The mucosa was moderately to severely infiltrated by heterophils, lymphocytes, and plasma cells. In the bronchi, similar lesions were seen but the epithelium was also often eroded or sloughed and, in some epithelial cells, a basophilic intranuclear inclusion was found (Figure 2). The plugs were

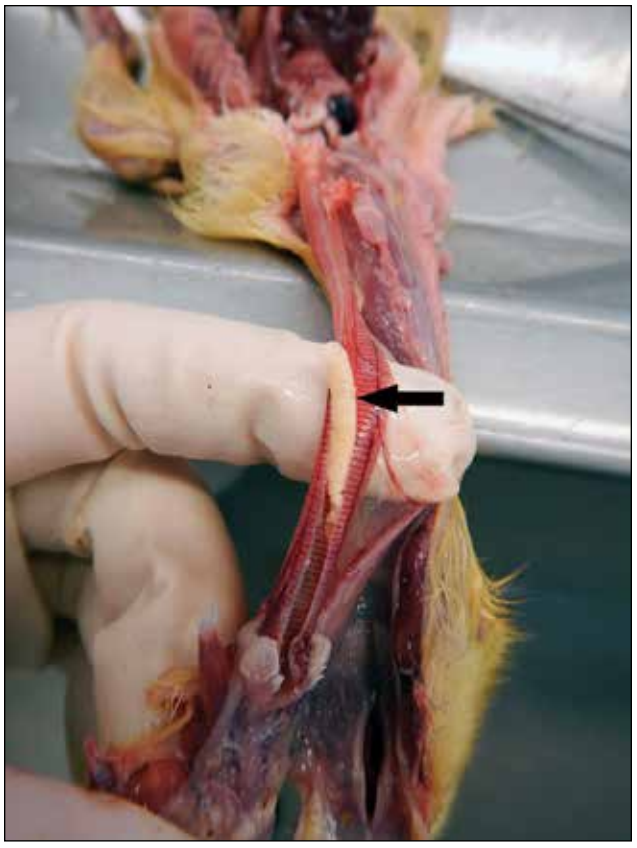

Figure 1. Trachea of an affected duckling. A white fibrin plug is completely obstructing the tracheal lumen.

composed of necrotic debris, sloughed epithelial cells, degenerated leucocytes, and a mixed population of bacteria. An heterophilic granuloma containing fungal hyphae (compatible with *Aspergillus*) was detected in an intrapulmonary bronchus of 1 bird. Samples of trachea and lungs were tested by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) for Newcastle disease (APMV-1) and influenza A viruses and were negative. Routine bacteriology of tracheal swabs and liver yielded contaminants only.

As part of the differential diagnoses, the presence of DAdV-1 was suspected and therefore, samples were submitted to the Molecular Diagnostic Laboratory (MDL) of the Faculté de médecine vétérinaire for analysis. No specific DAdV-1 PCR assay was available at MDL. Nonetheless, a pan avian adenovirus PCR assay, adapted from Raue et al (8), was available. Briefly, this PCR assay targets the hexon gene of avian adenoviruses and when positive, the expected PCR product length should be approximately 593 nucleotide base pairs. This pan avian adenovirus PCR assay was used on a pool of tracheal swabs, trachea, and lung. Interestingly, a PCR amplicon of the expected size was obtained, confirming the presence of an adenovirus. To identify the adenovirus species, the PCR product was sequenced using the Sanger method. Sequence analysis confirmed that adenovirus species was in fact DAdV-1 since the PCR product possesses a nucleotide identity of 99.63% compared to the DAdV-1 reference strain EDS76.

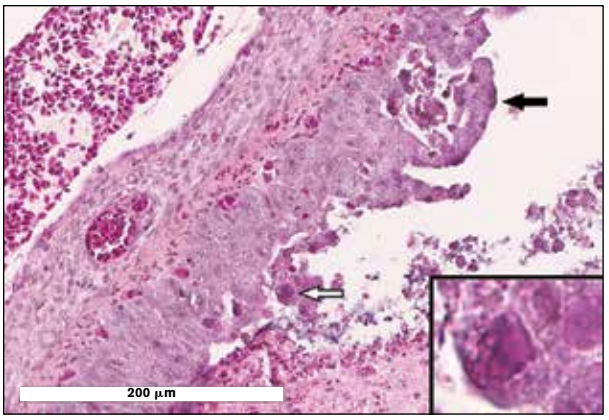

Figure 2. Bronchus of an affected duckling. There is hyperplasia and squamous metaplasia of the epithelium, infiltration by heterophils, and degeneration/necrosis and sloughing of individual to small groups of epithelial cells (black arrow). The lumen of the bronchus is filled with mucus, degenerated leucocytes, and necrotic epithelial cells and debris. The degenerated/necrotic epithelial cells contain a basophilic intranuclear inclusion body (white arrow and insert).

Considering that no entire viral genome sequence of DAdV-1 has been previously reported in Canada, we decided to establish whether our virus was similar to what has been previously reported worldwide. Thus, an attempt was made to sequence the entire viral genome directly from the clinical samples using MiSeq Illumina technology (Illumina, Vancouver, British Columbia) to allow us to better characterize our DAdV-1 strain, named FMV19-2234581. Briefly, the tissue was lysed for 10 min using glass beads. Then, the tissue lysate was centrifuged at low speed to remove large particles. The supernatant was placed on a sucrose cushion and then ultracentrifuged in an AirFuge microultracentrifuge (Beckman Coulter, Mississauga, Ontario) for 1 h to concentrate the virus. The pelleted virus was resuspended in phosphate-buffered saline (PBS) and the DNA was extracted using the Zymo RNA/DNA extraction kit (Cedarlane, Burlington, Ontario). The DNA was quantified using a Qubit fluorometer with the HS DNA kit, then a library was generated with a Nextera XT kit (Illumina). The library was sequenced using a MiSeq apparatus with a 600 v3 cartridge. The complete genome was obtained using the *de novo* metagenomic application plus the extend contigs option of the CLC Genomic Workbench, Version 12.0.3 software (Qiagen, Toronto, Ontario). The annotations were transferred using the reference strain Duck adenovirus 1 isolate D11-JW-032 (KJ452172) in Geneious Prime software, Version 2019.2.1 (Biomatters, Newark, New Jersey, USA). The nucleotide homology and PHYLM phylogenetic tree were made from a MAFFT alignment.

From the 9 674 970 total reads obtained, 0.025% (n = 2419) mapped to DAdV-1. A mean coverage of 16.07 (with a range of minimum and maximum values of 1 to 85) was obtained. The DAdV-1 FMV19-2234581 strain possesses a double-stranded DNA genome and was found to have a size of 33 222 nucleotides. The FMV19-2234581 nucleotide sequence was submitted to GenBank (accession number MN310513). The FMV19-2234581 whole viral genome nucleotide sequence clustered

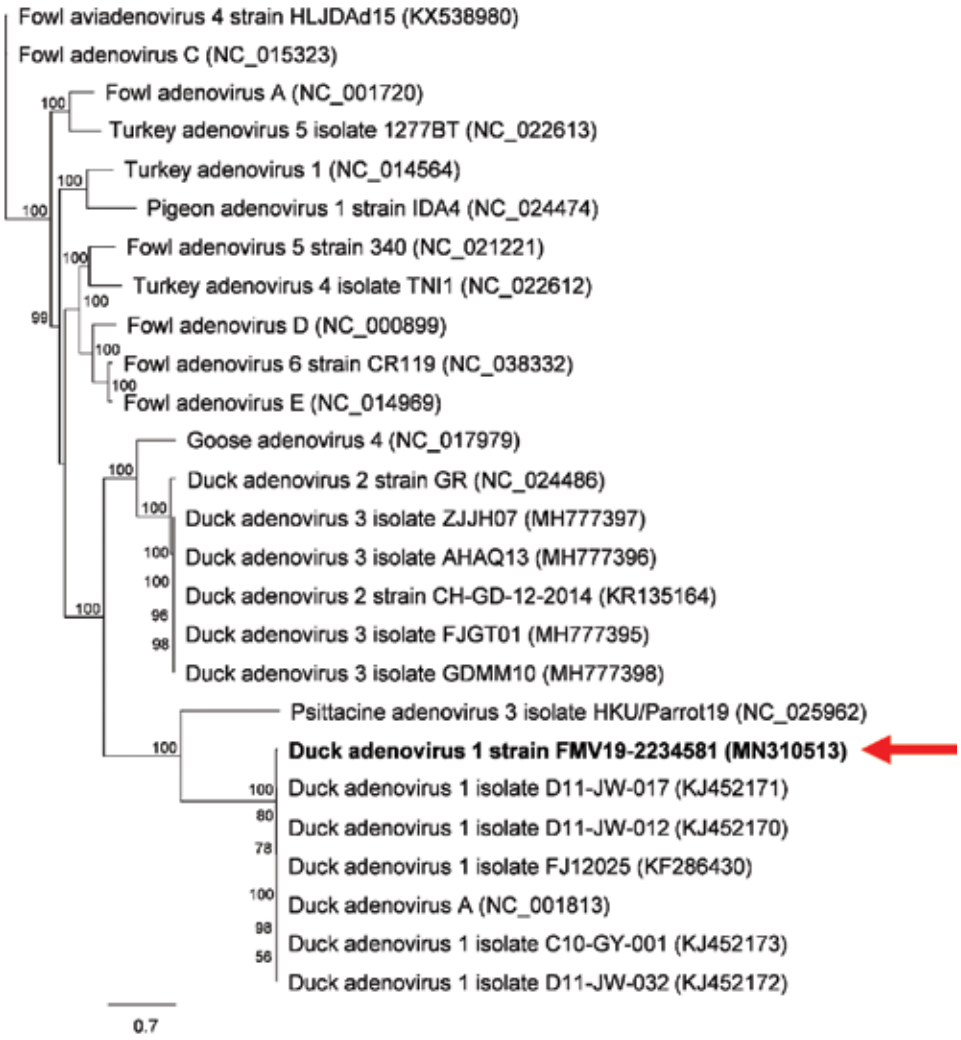

Figure 3. Nucleotide phylogenetic tree of the entire viral genome of different types of adenoviruses infecting various species of birds. Sequences of all the strains that are present in the tree were obtained from GenBank, their accession number is written following their name. The red arrow represents the complete viral genome of the first DAdV-1 Canadian strain to be sequenced (i.e., FMV19-2234581). The phylogenetic tree was created using nucleotide alignment PhyML Tree with substitution model HKY85 and 1000 bootstraps, using MAFFT alignment in Geneious Prime software version 2019.2.1. The bootstrap values are indicated in the tree. The scale bar represents the amount of nucleotide substitutions per site between viral strains.

with the other DAdV-1 reference strains as expected (Figure 3). Overall, the nucleotide identity between FMV19-2234581 and the other DAdV-1 reference strains varied between 98.23% and 98.33%.

After this outbreak, no new cases were reported in this group of ducklings and even when the producer bought other females throughout the year, no other groups of ducklings experienced clinical signs.

Discussion

This is the first outbreak of DAdV-1 tracheitis reported in Québec and only the second outbreak reported in Canada. It is unknown if this infection is common or not in commercial Canadian duck production because, to our knowledge, no extensive serologic survey for the detection of DAdV-1 antibodies has been carried out on these farms. In fact, the commercial DAdV-1 (Egg Drop Syndrome) ELISA test, designed for use in chickens, does not work on duck sera (Jantina De Vylder, Biochek, personal communication, 2019). In Europe, a hemagglutination inhibition test has been developed for use in ducks intended for export, but this test is not available in Canada (Hervé Morin, Filavie, personal communication, 2019). However, even if the virus is introduced into a flock, it seems moderately to weakly pathogenic because only 1 group of ducklings was affected at the farm, even though younger ducklings were housed on the same floor, with common ventilation and only separated from the affected group by a barrier. Thus, it is possible that this virus is more widespread than initially thought in commercial duck farms in Québec but remains undetected. Another interesting fact about this case is that only females

experienced clinical signs. This apparent sex-related sensitivity has never been reported in the literature. We could hypothesize that female ducklings responded differently to the virus when exposed to this agent for the first time. However, no females arriving at the farm after this episode became ill.

Interestingly, the FMV19-2234581 strain seems to be a more variable strain compared with other strains. In fact, the nucleotide identities of the other DAdV-1 strains varied between 99.738% and 100% when compared with each other, which is greater than when those strains are compared with FMV19-2234581 (98.234% to 98.327%). At present, it is difficult to establish if FMV19-2234581 could be a new virus subtype of DAdV-1 but further analyses will be required to determine this. All DAdV-1 strains included in the phylogenetic tree, however, originate from quite different geographical regions (i.e., China, Ireland, South Korea) compared with FMV19-2234581 (Canada). This geographical distance may have contributed to the increase in genomic evolution and diversity of the FMV19-2234581 strain compared with other DAdV-1 strains.

Acknowledgments

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