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Case report

Variant influenza A (H1N1) virus infection in Canada

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ABSTRACT

There has been an increase in influenza A variant detections in the US in recent years. In September 2012, an Ontario resident was diagnosed with influenza A (H1N1) variant infection. The demonstrated cross reactivity with the A(H1N1)pdm09 H1 gene CDC realtime PCR suggests that laboratories that only use the pdm09 H1 gene PCR to confirm this subtype would incorrectly report this variant as a A(H1N1)pdm09 subtype unless they were doing further molecular investigations.

1. Case description

There has been an increase in influenza A variant detections in the US in recent years.[1–3] Influenza viruses that normally circulate in swine are called "variant" viruses when they are found in people.[4] From December 2005 to September 21, 2012, 326 influenza A(H3N2) variant (v), 14 A(H1N1)v and 5 A(H1N2)v cases have been identified.[5] In September 2012, a 37 year old male Ontario resident was diagnosed with influenza A H1N1v infection. This was the first detection of this variant influenza strain in an individual in Canada. The case, who has an unknown influenza immunization history, had recently traveled within Canada during which time he had close contact with swine, and to the United States where he had close contact with cattle; attendance at swine fairs could not be ascertained. At the beginning of September, he became ill with gastrointestinal symptoms of unknown etiology, followed by acute respiratory symptoms and on September 9 was admitted to a local area hospital with pneumonia. Following a 2-day admission he was discharged from hospital, but was re-admitted on September 13 with worsening respiratory symptoms, and subsequently was transferred to a tertiary care center where he required admission to an intensive care unit. The case had two separate treatment courses of oseltamivir administered. The first course was for five days (75 mg twice daily) starting on September 16, 2012. He had a second seven-day course of 150 mg twice daily starting on September 21, 2012. At time of writing the case had been discharged home following an extended stay in intensive care, however additional travel and exposure information was unattainable. Only one close contact of the case reported symptoms of influenza-like illness (ILI); that person tested negative for influenza.

2. Laboratory testing

PHOL performs a large proportion of primary respiratory viral testing for the province of Ontario from a variety of clinical settings including ambulatory, hospital and outbreaks. Specimens from all hospitalized and outbreak patients are tested for influenza A matrix gene and influenza B NS1 gene using CDC protocols. If influenza A-positive, subtyping for seasonal influenza A (H3N2) HA gene (CDC assay) and influenza A (H1N1)pdm09 NA gene (in-house assay) are performed.[6] Following the emergence of H3N2v infections in the US in 2011, PHOL implemented screening of a selection of influenza A-positive, H3-positive specimens with the swine NP gene PCR to screen for H3N2v. Samples that are influenza-A positive, but negative in the initial subtyping assays are designated unsubtypeable, and are investigated further including other seasonal (N2, H1, N1, pdm09H1), swine (NP) and avian (H5, H7, +/− H9) rRT-PCR subtyping targets using CDC protocols, in addition to an end-point swine influenza matrix (M) gene PCR developed by Canada's National Microbiology Laboratory (NML). Influenza-negative outbreak and ICU samples are also tested using

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Table 1
Influenza A virus with highest nucleotide sequence identity to A/Ontario/N163578/2012 influenza virus (HINIv) isolated in Ontario as determined by a blast search in GenBank.

	Gene segment	Nucleotide sequence identity (%)	Subtype	Virus with the highest identity
1	PB2	98.90	H1N1	A/swine/Illinois/A00857129/2011
2	PB1	99.26	H3N2	A/swine/Illinois/A00857318a/2012[a]
3	PA	99.35	H3N2	A/swine/Illinois/A00857318a/2012[a]
4	HA	99.24	H1N1	A/swine/South Dakota/7/2011
5	NP	99.20	H3N2	A/swine/Indiana/A01049091/2010
6	NA	98.92	H1N1	A/swine/Minnesota/A01134524/2011
7	M	99.49	H3N2	A/swine/Illinois/A00857318a/2012[a]
8	NS	99.64	H3N2	A/swine/Illinois/A00857318a/2012[a]

[a] Triple-reassortant virus (trH3N2) containing M and NP genes of pandemic H1N1.

a multiplex molecular respiratory viral assay [Seeplex RV15 ACE detection kit (Seegene, Inc., Seoul, South Korea) (MRVP)]. Assay targets include rhinovirus, enterovirus, influenza A/B, parainfluenza 1–4, respiratory syncytial virus A/B, adenovirus, bocavirus, human metapneumovirus, coronavirus OC43, coronavirus 229E/NL63 and bocavirus. Respiratory specimens from ambulatory and emergency patients not admitted to hospital are cultured for virus isolation in two cell lines. One line is monkey kidney cells [either Rhesus (RMK) or African green monkey kidney cells (AGMK)], and the other is WI-38 human embryonic lung fibroblast cells (Diagnostic Hybrids, Inc., Athens, OH, USA). A selection of influenza A-positive cultures are subtyped as described above.

A nasopharyngeal swab from the case collected on September 10 tested positive for influenza A in viral culture, and specimens collected on September 12 and 15 during a bronchoalveolar lavage was also positive for influenza A in the MRVP assay. A subsequent nasopharyngeal swab collected on September 17 tested negative for influenza. The September 10 sample was initially unsubtypeable by the influenza A subtyping assays routinely used at PHOL – influenza A(H1N1)pdm09 NA gene and seasonal HA3 gene. Real-time subtyping PCRs for seasonal HA1, seasonal NA1, seasonal NA2, HA5, and HA7 were all negative. Real-time PCRs for swine nucleoprotein (NP) gene and A(H1N1)pdm09 HA1 gene were both positive, as was a PCR for swine M gene. These results suggested that the influenza A virus was of swine origin, and of H1 subtype.

Whole genome sequencing was commenced at PHOL, and sequence data shared with the National Microbiology Laboratory (NML), Canada's reference laboratory. The NML received the primary sample and culture material for further investigation. At NML, testing was performed on the culture material, which was negative for seasonal H3N2, pandemic NA, H3N2 variant (v), and H1N2v. Antiviral susceptibility testing showed that the isolate was resistant to amantadine but sensitive to oseltamivir and zanamivir. Complete sequencing analysis of PB1, PB2, PA, HA, NA, and M genes on viral RNA extracted from the first passage in rhesus monkey kidney cells revealed that all sequences are very similar (between 98.9 and 99.5%) to viruses that have been found in US swine in 2011 and 2012 (Table 1). Interestingly, four of the internal genes (PB1, PA, NP and NS) shared the highest homology of 99.26–99.64% to A/swine/Illinois/A00857318a/2012, a swine triple reassortant H3N2 isolate containing M and NP genes of pandemic H1N1.

The sequences for A/Ontario/N163578/2012(H1N1v) were deposited in the GISAID database under accession numbers EPI397237–EPI397244. PCR and sequencing results of HA and NA showed that this virus is an H1N1 subtype, which is closely related to H1N1 swine triple reassortant viruses identified in the United States in 2011 and 2012. Sequencing revealed that this H1N1 carries the M gene of influenza A(H1N1)pdm09, as was found in the last 2 documented cases of H1N1v infection, in Wisconsin (December 2011) and Missouri (reported September 2012). The nucleotide and deduced amino acid (aa) sequences comparison of the HA gene of A/Ontario/N163578/2012 revealed 99.7% nucleotide (100% aa) and 97.1% nucleotide (97.2% aa) homologies to HAs of the two most recent H1N1v cases in Wisconsin and Missouri. However the HA nucleotide homology was found to be lower at 92.7% (90.8% aa homology) to A/California/7/2009 (H1N1)-like virus, the A(H1N1)pdm09 strain in the current seasonal influenza vaccine (Table 2). The even lower nucleotide homology to A/California/7/2009 (H1N1)-like virus NA gene (79.6%) is consistent with this virus not being detected by the PHOL in-house A(H1N1)pdm09 NA gene real-time PCR due to several scattered mismatches in primer and probe binding sites.[6]

Comparison of the HA1 gene of the Ontario influenza A (H1N1) variant with the reference vaccine strain A/California/07/2009 revealed 30 amino acid substitutions including: D35N, K36R, I61L, P83S, S84N, D97N, S128T, K130R, A141T, L151I, V152A, L161I, S162N, D168N, G170E, G178A, K208R, K211E, I216T, E224A, V250A, M257L, E258K, A261S, P271S, N276D, K283N, I298V, K302E, L314M. Among these substitutions, 5 are located in antigenic sites Ca (A141T, D168N, G170E) and Sa (L161I, S162N). Convalescent serum collected three weeks after illness onset (September 26th 2012) had hemagglutination inhibition (HI) titers of 20, <10, and 40, respectively, against reference vaccine strains, A/Perth/16/2009 (H3N2), B/Brisbane/60/2008, and B/Wisconsin/1/2010. However, it had a very high titer of 5120 against the vaccine strain A/California/7/2009 (H1N1). Antigenicity of the H1N1v isolate was examined using ferret antisera to A/California/7/09 by HI assay; it cross-reacted with an HI titer of 160 (reference HI titer is 640). This serological profile suggests close antigenic homology between A(H1N1)pdm09 and the H1N1v strain reported here. Based on the testing results from PHOL and NML, we concluded that this patient was infected with an influenza A (H1N1)v virus (Table 3).

3. Discussion

Based on data from the US Centers for Disease Control and Prevention, two A (H1N1)v viruses (A/Wisconsin and A/Missouri) isolated from humans in the US in 2011 and 2012, are closely

Table 2
Nucleotide (amino acid) homology comparison of HA1 and NA gene among recent A(H1N1) variants and A/California/07/2009 vaccine strain.

	A/California/07/2009		A/Ontario/N163578/2012		A/Missouri/12/2012		A/Wisconsin/28/2011	
	HA1	NA	HA1	NA	HA1	NA	HA1	NA
A/California/07/2009			92.7 (90.8)	79.6 (83.9)	92.9 (90.8)	79.5 (83.9)	92.1 (89.9)	79.4 (82.8)
A/Ontario/N163578/2012	92.7 (90.8)	79.6 (83.9)			99.7 (100)	99.4 (99.3)	97.1 (97.2)	96.8 (95.6)
A/Missouri/12/2012	92.9 (90.8)	79.5 (83.9)	99.7 (100)	99.4 (99.3)			97.2 (97.2)	97.3 (96.3)
A/Wisconsin/28/2011	92.1 (89.9)	79.4 (82.8)	97.1 (97.2)	96.8 (95.6)	97.2 (97.2)	97.3 (96.3)		

Table 3
Summary of sample type received at PHOL and tests performed for H3N2v case.

Type of sample	Date of sample collection	Result
Nasopharyngeal swab #1	September 10, 2012	Culture results: positive for influenza A rRT-PCR*: positive for influenza A, but unsubtypeable Multiplex respiratory viral PCR (MRVP) testing: not performed
Bronchoalveolar lavage #1	September 12, 2012	Culture results: results not available rRT-PCR testing: influenza A matrix gene, pandemic H1 detected; N1, N2, seasonal H1, H3, H5, H7 not detected MRVP: positive for influenza A
Bronchoalveolar lavage #2	September 15, 2012	Culture results: not tested by culture-based methods. Tested by MRVP rRT-PCR testing: influenza A matrix gene, swine NP, pandemic HA1 detected; N1, N2 seasonal H1, H3, H5, H7 not detected MRVP: positive for influenza A
Nasopharyngeal swab #2	September 17, 2012	Culture results: not tested by culture-based methods rRT-PCR testing: not performed MRVP: influenza A not detected

related antigenically to A/California/7/2009 virus, which is a vaccine component of the currently available influenza vaccine. It is expected that the 2012–2013 seasonal influenza vaccine will confer protection against the A (H1N1)v influenza virus, although the seasonal influenza vaccine may not protect people against some other variant influenza viruses that are very different from currently circulating human influenza viruses. This study also confirms occurrence of an A (H1N1)v infection in Canada with a strain that is genetically very closely related to the two recent A (H1N1)v strains from Wisconsin and Missouri. This suggests a common origin and geographic spread from the United States into Canada. It also raises the possibility of recent human A(H1N1)v infections that have gone undetected in the US and Canada. The demonstrated cross reactivity with the A (H1N1)pdm09 H1 gene CDC realtime PCR suggests that laboratories that only use the pdm09 HA1 gene PCR to confirm this subtype would incorrectly report this variant as a A (H1N1)pdm09 subtype unless they were doing further molecular investigations. This highlights the need for ongoing influenza surveillance, including subtyping, strain typing, and molecular characterization and the need for a representative selection of influenza viruses to have further molecular investigation by sequencing, which would ultimately detect any significant circulation of such variants. Such infrastructure will also enable rapid detection of more virulent influenza viruses, such as highly pathogenic avian influenza A (H5N1).

We also suggest that a representative sample of influenza viruses from a variety of settings (e.g. critical care, outbreak-related, community-based) be screened by molecular methods in order to detect variants. Following the emergence of H3N2v infections in the US in 2011, PHOL does screen a selection of influenza A, subtype H3 specimens, with the swine NP gene PCR to screen for H3N2v. However, the NP gene PCR is positive in influenza A (H1N1)pdm09 as well as H1N1v, as both are of swine origin. In general, exposure and travel-related information is poorly completed on laboratory requisitions, and not consistently asked of patients presenting with influenza-like illness, particularly during influenza season, hence many variant cases would be missed. It is anticipated that finding a variant virus would be very rare, thus it is not essential to test all patients for both the HA and NA genes of influenza A (H1N1)pdm09. However systematic sequencing, which is primarily conducted in order to detect changes in the influenza genome which could impact vaccine effectiveness, could optimize detection of variants. This work should be conducted by reference laboratories as part of influenza surveillance.

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Competing interests

Jonathan B. Gubbay has received a research grant from GlaxoSmithKline Inc. to work on resistance to neuraminidase inhibitors. In June 2010, Public Health Ontario received a research grant from GlaxoSmithKline to study phenotypic resistance in the influenza virus.

Ethical approval

Research ethics approval was not required.

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