A variety of respiratory viruses found in symptomatic travellers returning from countries with ongoing spread of the new influenza A(H1N1)v virus strain

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Clinical specimens from 79 symptomatic individuals with a recent history of travel to countries with verified transmission of influenza A(H1N1)v (North America) were tested with a multiple real-time PCR targeting a broad range of agents that may cause acute respiratory infection. This analysis revealed that besides four cases of influenza A(H1N1)v, other respiratory viruses were diagnosed in almost 60% of the samples. These observations are a reminder that many different viral transmissions occur simultaneously in countries with ongoing spread of influenza A(H1N1)v. The findings demonstrate that the definition of suspected cases by clinical and epidemiological criteria has only a poor capacity for discriminating influenza A(H1N1)v from other viral infections.

Background

A new influenza A(H1N1)v variant has spread globally since its first appearance in April 2009 [1,2] and as of 17 June 2009 there were 39,620 cases reported by the World Health Organization (WHO) [3]. On 30 April 2009, the European Commission suggested a case definition [4], which has been adopted and modified by most authorities in the European Union Member States. In agreement with this recommendation, testing for influenza A was recommended in Sweden for cases with a clinical presentation including respiratory symptoms and fever above 38°C, and epidemiological circumstances such as recent travel (within seven days) to areas where the new influenza has been observed [5] or close contact with confirmed cases.

The regular sentinel surveillance for seasonal influenza has been extended and now focuses on identification of imported cases with influenza A(H1N1)v, and on preventing secondary transmission by contact tracing and antiviral medication in an attempt to delay sustained community transmission. In order to provide a better basis for the decision whether or not to initiate preventive measures, expanded testing, targeting a broad range of respiratory agents, has been applied to specimens from all suspected cases in the region Västra Götaland (1.5 million inhabitants). We report here the results of this expanded testing.

Material and methods

This report includes samples of patients who, during the period from 24 April to 10 June 2009 presented with influenza-like symptoms and a history of recent travel to the United States or

TABLE 1

Primers and probes for typing of influenza A virus by real-time PCR run in three parallel reactions

Oligonucleotide primers*	Sequence
IAH1_F	CYGACACTGTTGACACAGTACTTGAGA
IAH1_R	CGGCAACGCTGCAATTACC
IAH1_Probe	TGACAGTGACACACTCTGTCAACCTACTTGAG
IAH3_F	GCAACTGTTACCCTTATGATGTGC
IAH3_R	CATTGATAAACTCCARRGTGCCKGA
IAH3_Probe	ATGCCTCCCTTAGGTCACTAGTTGCCTC
IAH1v_F	GGGGTAGCCCCATTGCATT
IAH1v_R	GTGGAGAGTGATTCACACTCTGGA
IAH1v_Probe	CCCAGGATCCAGCCAGCAATGTTACA

* The oligonucleotide primers target type-specific regions of the haemagglutinin gene, and IAH1v oligonucleotides are specific for the new influenza A (H1N1)v variant.
Y: C/T mixture; R: G/A mixture.

TABLE 2

Viral aetiologies for the patients fulfilling definition of suspected cases of influenza A(H1N1)v, region Västra Götaland, Sweden, April-June 2009 (n=79)

Viral aetiology	Number	Percentage (%)
Rhinovirus	28*	34
Coronavirus	8	10
Influenzavirus B	3	4
Human parainfluenzavirus (1-3)	3	4
Adenovirus	2*	2
Influenzavirus A(H1N1)v	4	5
Metapneumovirus	1*	1
Enterovirus	1*	1
Respiratory syncytial virus	0	0
Mycoplasma pneumoniae, Chlamydia pneumoniae	0	0
Negative	32	39
Total number	82*	100 %

* Three patients had double infections with rhinovirus together with enterovirus, metapneumovirus or adenovirus.

Mexico, and therefore were recommended for examination and sampling. This clinical examination was performed by infectious disease clinicians on call at Sahlgrenska University Hostpital/ Östra in Gothenburg, and our report is based on their evaluation and laboratory results. In summary, of all 79 patients included with a travel history, 90% presented with respiratory symptoms, 5% without respiratory symptoms, and for the remaining 5% this information is not documented. Sixty-six percent had fever above 38°C, 29% had no fever, information on fever was missing for 5%. Nasopharyngeal swabs were sent to the molecular diagnostic unit at the virological laboratory at Sahlgrenska University Hospital for testing by a multiple real-time PCR targeting 13 viruses and two bacteria, run in six parallel multiplex PCRs on an ABI 7500 instrument [6]. Samples that were reactive for the influenza A component (matrix protein target, [7]) of this PCR were subtyped by an additional real-time PCR targeting the haemagglutinin gene, run in three parallel reactions specific for the H3N2 and H1N1 subtypes that have been circulating for a long time, as well as for the new H1N1v strain (Table 1).

Results and discussion

In total, samples from 79 patients were tested (42 males, 37 females; median age 30 years, range 1-75 years), with between 10 and 16 samples on average each week and most of them taken from patients with respiratory symptoms and a history of recent travel to North America (Figure). Four cases with the new influenza A (H1N1)v variant were diagnosed. Interestingly, in 56% of the cases, other aetiologies were identified (Table 2).

The most common finding was rhinovirus, observed in 28 of 82 cases (34%) and three of these patients also had a second viral infection (enterovirus, metapneumovirus and adenovirus). The frequent identification of rhinovirus and other viruses demonstrates that the criteria for suspected cases of influenza A(H1N1)v are relevant as indicators of a viral infection, but not specific for influenza A. On the other hand, applying more restrictive criteria would probably have excluded most infections with the new A(H1N1)v strain, considering that their clinical presentation has been reported to be relatively mild. This illustrates a dilemma with surveillance actions aiming at revealing the spread of new respiratory infections. If the applied criteria are too strict (for example fever above 39°C, cough and muscle pain), the epidemic is likely to be underestimated, because only the severe cases are identified. If on the other hand the criteria are liberal, as illustrated by the current epidemic, most of the cases will probably have other aetiologies. The positive predictive value of clinical criteria for identification of influenza A is particularly low in the early phase of an epidemic, when the incidence of influenza A is low, but will become relatively high during the peak when a large proportion of respiratory infections will be due to influenza A virus. The value of broad virology testing decreases in the course of an influenza epidemic, when the detection rate of other aetiologies may decrease from above 50% as observed in this report to below 10% during the influenza peak (unpublished observations from our laboratory).

The cases with influenza A were analysed further by a typing PCR that within 4-5 hours could identify whether the strain was a traditional H1N1 or H3N2 virus, or the new H1N1 variant. This typing system targets specific regions of the haemagglutinin gene and has been developed in our laboratory (unpublished). It has proved to have a good sensitivity, as illustrated by cycle threshold (Ct) values that are typically lower than those obtained in the general PCR for influenza A, which targets a conserved region of the matrix protein gene.

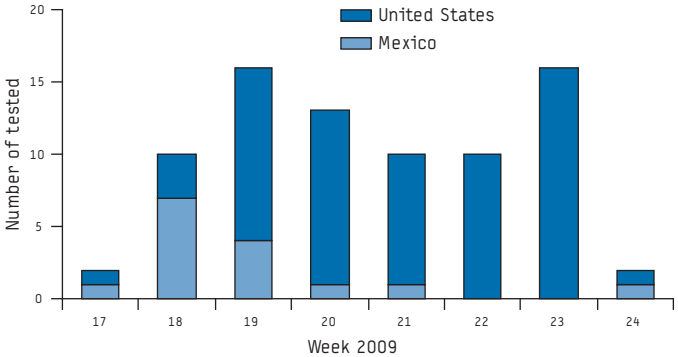
The results of the multiple PCR used in our setting were available within 24 hours after sampling and served at the same time as confirmation for the result of the first, general influenza A PCR. In cases that presented with typical influenza-like symptoms but were negative for influenza A in the first PCR, the finding of an alternative aetiology was helpful for the decision to refrain from preventive measures. Such measures include oseltamivir treatment of patients and influenza testing and prophylactic treatment of their close contacts. The clinical practice was not always different, but in some cases the identification of an alternative aetiology such as rhinovirus was helpful for the decision not to treat the patient of contacts, even when the patient had symptoms clearly indicative of possible influenza. From this experience we therefore conclude that a broad diagnostic test is a valuable tool in the early investigation of a new emerging respiratory virus like the new influenza A(H1N1)v.

Note added in proof:

On 17 June, Sweden changed to a stricter case definition for suspected cases. It now requires more than two symptoms besides epidemiology and fever.

Figure

Individuals tested each week, region Västra Götaland, Sweden, April-June 2009 (n=79)



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