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Transmission potential of the new influenza A(H1N1) virus and its age-specificity in Japan

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On 16 May 2009, Japan confirmed its first three cases of new influenza A(H1N1) virus infection without a history of overseas travel, and by 1 June, 361 cases, owing to indigenous secondary transmission, have been confirmed. Of these, 287 cases (79.5%) were teenagers (i.e. between 10 and 19 years of age). The reproduction number is estimated at 2.3 (95% confidence interval: 2.0, 2.6). The average number of secondary transmissions involving minors (those under 20 years of age) traced back to infected minors is estimated at 2.8. That is, minors can sustain transmission even in the absence of adults. Estimates of the effective reproduction number R_t moved below 1 by 17 May. Active surveillance and public health interventions, including school closures most likely have contributed to keeping R_t below one.

Introduction

The reproduction number *R*, the average number of secondary cases generated by a single primary case, of the new influenza A(H1N1) virus, is a key quantitative measure for assessing pandemic potential [1]. In the ongoing epidemic of the new influenza A(H1N1) virus, early studies suggested that *R* ranged from 1.4-1.6 [2] and some estimated it to be as high as 2.2-3.1 [3]. Estimates in 1.4-1.6 range for the new influenza A(H1N1) virus are lower than estimates based on data from, for example, the fall wave of the 1918 influenza pandemic [4,5]. The present study investigates indigenous secondary transmissions of the new influenza A(H1N1) virus in Japan, not only estimating *R* but also exploring its age-specificity.

Methods

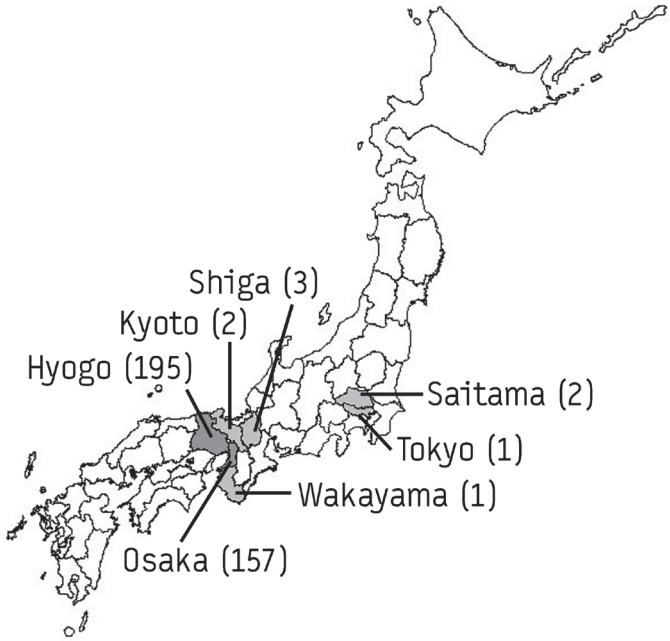
Epidemiological description of the epidemic

On 16 May 2009, three high school students in Kobe city, Hyogo prefecture, without a history of overseas travel, were confirmed as infected with the new influenza A(H1N1) virus. Confirmatory diagnosis in Japan requires influenza-like symptoms and a laboratory diagnosis which is made either by virus isolation, real-time PCR or a significant increase in neutralising antibody titre against the virus. Further confirmed diagnoses followed predominantly in Hyogo and Osaka prefectures. The increased number of infections among particular age groups was most evident in the data from prefectures where most secondary cases were found among high school students attending different schools.

By 1 June, the Ministry of Health, Labour and Welfare of Japan had reported 371 confirmed cases, including nine imported cases and one case traced back to a distant international airport (i.e. a worker at Tokyo-Narita airport) [6]. Figure 1 shows the geographic distribution of 361 indigenous cases. Cases outside Osaka and Hyogo prefectures had travel histories to Osaka or Hyogo before their illness onset. The index case(s) (who may have remained asymptomatic [7]), with a history of overseas travel, has (have) yet to be identified. Furthermore, there are no known cases prior to the five confirmed cases that developed the disease on 9 May in Hyogo (Figure 2A). The triggering event may be associated with Japan's two-week festive break, the "golden week", just before 9 May, when people may have travelled to and returned from Mexico, United States and Canada.

Figure 1

Spatial distribution of the epidemic of new influenza A(H1N1) virus infection in Japan. Cumulative number of confirmed indigenous cases, as of 1 June 2009 (n = 361)



Note: Cases in Tokyo, Saitama, Shiga and Kyoto had travel history to either Hyogo or Osaka prefecture before illness onset. Kobe city, where first three cases were diagnosed, is a capital city of Hyogo prefecture.

We analysed the temporal incidence distribution of confirmed cases for this epidemic (Figure 2A). The known dates of illness onset are used except for a fraction of the confirmed cases in Kobe city (45; 12.5%) whose dates of onset have yet to be fully clarified. Since the known median time from onset to diagnosis in Kobe has been estimated at 1.0 day [8], it is assumed that the dates of onset among the 45 cases in Kobe were 1 day before their date of diagnosis. We observed that by the time the first three cases had been confirmed (16 May), the epidemic curve was just about at its peak. 16-17 May fell on a weekend, and all schools in Osaka and Hyogo were officially closed for one week starting on 18 May. Figure 2B displays the age-distribution of the 361 confirmed cases, which is concentrated in the teenage population. We see the age-specific window (10-19 years of age) that includes 287 confirmed cases (79.5%; 95% confidence interval (CI): 75.3, 83.7).

Epidemiological analysis

Taking into consideration the high levels of uncertainty related to the invasion of a population by a novel influenza virus, three different methods are used to estimate the transmission potential of the new influenza A(H1N1) virus. To concentrate on the transmission potential in Japan, all nine imported cases and one case that is not associated with indigenous transmission in Hyogo and Osaka were removed from the following analyses.

Model 1 (M1)

Estimation of R using the intrinsic growth rate [3,5]. The intrinsic growth rate r, is estimated via a pure birth process [9]. The likelihood is proportional to:

$$\exp\left(-r\sum_{i=0}^{t-1}C(i)\right)\left(1-\exp(-r)\right)^{C(t)-C(0)}$$

where C(t) denotes the cumulative number of cases on day t. $C(0) = 5$ and t = 0 represents 9 May. The generation time (GT) is assumed to follow a gamma distribution with mean $\mu = 1.9$ days and coefficient of variation $v = 47\%$ [2]. R is subsequently estimated using the estimator [10]:

$$(1+r\mu v^2)^{\frac{1}{v^2}}$$

Given that many serial intervals reported from Spain are longer than 1.9 days [7], the uncertainties surrounding GT estimates are partially addressed through a sensitivity analysis of R to variations in the mean GT in the range from 1.3-4.0 days. The exponential growth phase is assumed to have a mean duration of 8 days but windows in the 8±2 days were also used.

Model 2 (M2)

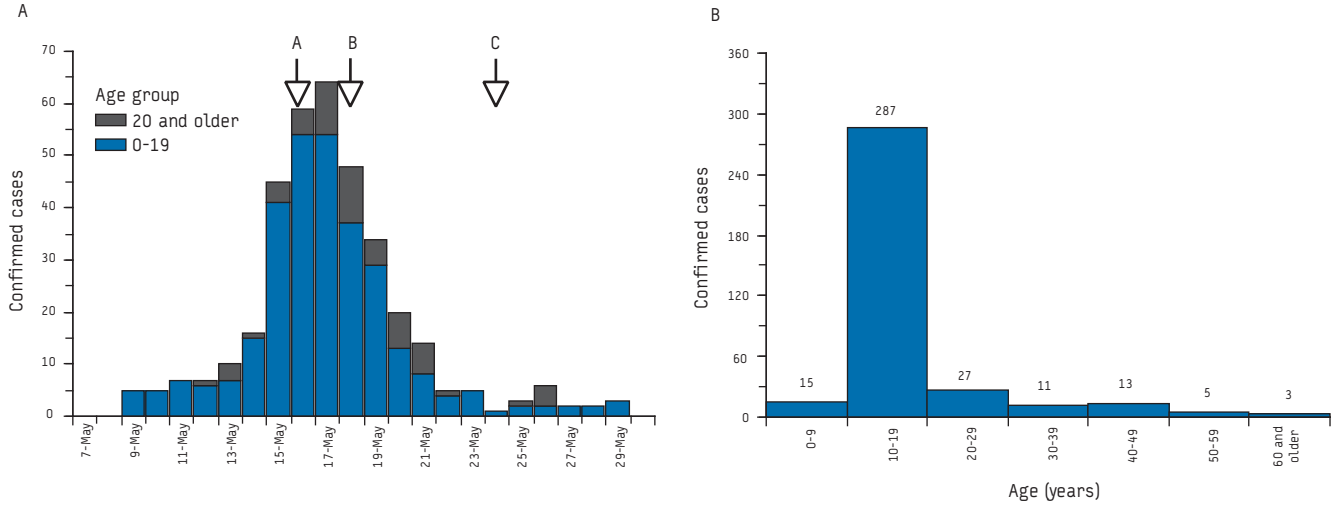
The effective reproduction number R_t, the average number of secondary cases generated by a primary case at time t, is estimated. The daily growth rate rt is used to estimate R_t following the approach described elsewhere [11]; the distribution of GT and the estimator of R used are the same as those used in M1. The mean GT is assumed to be 1.9 days but varying in the 1.3 to 2.5 days range [2].

Figure 2

Time- and age-specificity of the epidemic of new influenza A(H1N1) virus infection in Japan

A) Epidemic curve of confirmed indigenous cases according to the date of illness onset, as of 1 June 2009 (n = 361)

B) Age distribution of confirmed indigenous cases, as of 1 June 2009 (n=361)



Note: None of the confirmed cases had recent history of overseas travel (except for one case in Wakayama). The nine cases, believed to have become infected abroad, and one case, arising in a worker at Tokyo-Narita airport, are excluded from these figures.
The dates of illness onset for each confirmed case are reported by prefectural governments, except for a fraction of cases in Kobe city where cases with unknown dates of onset are assumed to have developed the disease one day before the confirmatory diagnosis (based on published median estimate [8]).
It should be noted that the dates of onset are based on preliminary reports and have yet to be refined.
Arrow A indicates the date on which the first three cases were diagnosed in Kobe city. All schools in Hyogo and Osaka were closed between the dates signalled by the arrows B and C.

Model 3 (M3)

The role of age-specificity in transmission is analysed using estimates of the next-generation matrix, K (Figure 3). First, we aggregate the population in two age groups, minors and adults. Second, since the mean GT is approximately 2 days [2], the daily number of cases during the exponential growth phase (i.e. first 8 days) uses as its unit of time, two-day intervals (i.e. cases, c, in days 1 & 2, 3 & 4, 5& 6 and 7 & 8 are grouped). Third, the expected value of cases in age-group i of grouped-generation τ, $E(c_i(\tau))$, is modelled by $R_{ii}c_i(\tau\text{-}1)+R_{ij}c_j(\tau\text{-}1)$ (fort = 2, 3 and 4) where R_{gh} is the element of K that corresponds to the average number of secondary cases in group g caused by an infected individual in group h. We estimate the entries in the matrices, assuming two different mixing patterns modelled via two unknown parameters by means of Poisson regression (Figure 3).

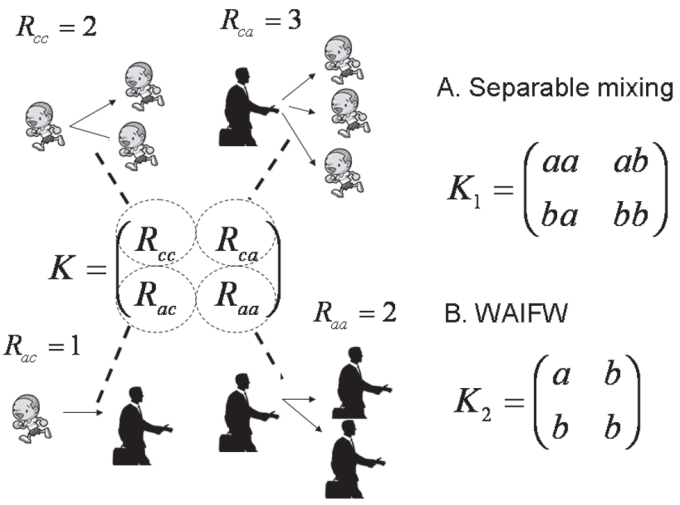
Results

The intrinsic growth rate r, is estimated at 0.47 (0.40, 0.56) per day. Accordingly, M1 gives an R estimate of 2.3 (95% CI: 2.0, 2.6). Figure 4A illustrates the sensitivity of R to variations in the mean GT in the range 1.3-4.0 days. The corresponding R estimates lie in the 1.8 to 4.8 range. Variations in the initial growth phase (i.e. ±2 days) do not greatly influence R; i.e. the expected values of R lie in the 1.9 to 2.3 range. The exclusion of the less documented cases in Kobe lead to an R estimate of 2.0 (95% CI: 1.7, 2.3).

Use of M2 suggests that R_t peaked on 14 May (Figure 4B). On 17 May, the day after a press release announced the first three confirmed diagnoses, R_t declined below 1. Under active surveillance efforts and school closures, R_t was kept below 1 thereafter. Consistent temporal patterns of R_t are seen using different values, except for slight increase and decrease in R_t estimates, for GT mean values in the 1.3-2.5 day-range.

FIGURE 3

Next-generation matrix



Note: Each element of the next-generation matrix, i.e., R_{cc}, R_{ca}, R_{ac} and R_{aa}, denotes the average number of secondary transmissions caused by a single primary case for child-to-child, adult-to-child, child-to-adult and adult-to-adult transmissions, respectively (note that here "child" represents "minor", aged from 0 to 19 years). The reproduction number R, for the whole population, is given by the largest eigenvalue of the next-generation matrix. By making qualitative assumptions A and B, two parameters, a and b, are estimated.

Using M3, the next-generation matrix, K_1 estimate, under the separable mixing assumption is

$$\hat{K}_1 = \begin{pmatrix} 2.82 & 0.32 \\ 0.32 & 0.04 \end{pmatrix}$$

while our K_2 estimate based on a qualitative assumption of WAIFW (who acquired infection from whom) matrix is

$$\hat{K}_2 = \begin{pmatrix} 2.82 & 0.29 \\ 0.29 & 0.29 \end{pmatrix}$$

The host-specific reproduction number [12] for minor, i.e. the average number of secondary minor cases generated by a single primary minor case was 2.8 under K_1 and K_2. Hence a population of minors can sustain the chains of secondary transmission even in the absence of adults (i.e. for this epidemic "minors" are the "core" group). Our estimate of R based on M3 is the largest eigenvalue of K, and R is estimated at 2.9 for both matrices. These estimates are slightly greater than R estimates based on M1; when the mean and variance of GT is 2.0 days and 0 days^2 (i.e. if GT is constant, following a delta function), our R estimate is 2.6.

Discussion

Two important conclusions can be drawn from our epidemiological analyses. Firstly, the reproduction number R of the new influenza A(H1N1) virus in Japan is estimated to be as high as 2.3, a value that is significantly higher than that recently reported [2]. The pandemic potential of this virus in Japan may be higher in terms of transmission potential than in other areas of the world. In particular, it should be noted that our estimate of R is greater than published estimates for seasonal influenza epidemics in temperate countries [13]. Given that our R estimate has been tested for robustness to uncertainty to mean GT, it seems plausible that high contact rates among teenagers (when compared to other populations) may be one of the main drivers of this epidemic. From a transient increase in R_t around 14 May, our high estimate of R may reflect the existence of few highly connected clusters of cases among "cliques" of high school students. There may be additional contributing factors to variations in our R estimates, including cross-protective immunity due to previous exposure to other closely related influenza viruses.

Secondly, our age-specific estimates support the view that minors can sustain transmission of the new influenza A(H1N1) virus among themselves. Available data are not enough to investigate the precise role of age-specific effects (e.g. different roles of transmission among infants, primary-school, high-school and university students) due to small case counts. Nevertheless, we believe that the population of minors could play a key role as a "reservoir" for sustained chains of secondary transmission, despite the fact that cases in this group include those infected in some atypical school clusters. Should further data confirm these results then the value of public health interventions targeting minors (closing schools and further contact restrictions between minors) could be effective in controlling further outbreaks in Japan and other countries.

Our estimates of R_t provide a quantitative measure of the time-evolution of the "force" of the epidemic. Although the dates of onset have yet to be refined and, thus, the precision of R_t estimate may have been influenced by possible delay in diagnosis and reporting, R_t declined below 1 one day after the news of the first

three confirmed diagnoses. Thereafter, the implementation of active surveillance programmes, including contact tracing, combined with school closures, most likely have contributed to keeping R_t below 1.

R is useful for assessing transmission potential, and it is one of the ways of assessing pandemic potential. This study puts emphasis on quantifying the impact of contact patterns on the transmission potential, factors that vary across space and time. Thus, further analyses of *R* for the new influenza A(H1N1) virus in different settings are needed to better quantify the role of uncertainty and heterogeneous patterns of transmission in these estimates. Validation of our quantitative understanding of the role of age-specific transmission should lead to improved effectiveness of age-specific control measures.

Acknowledgements

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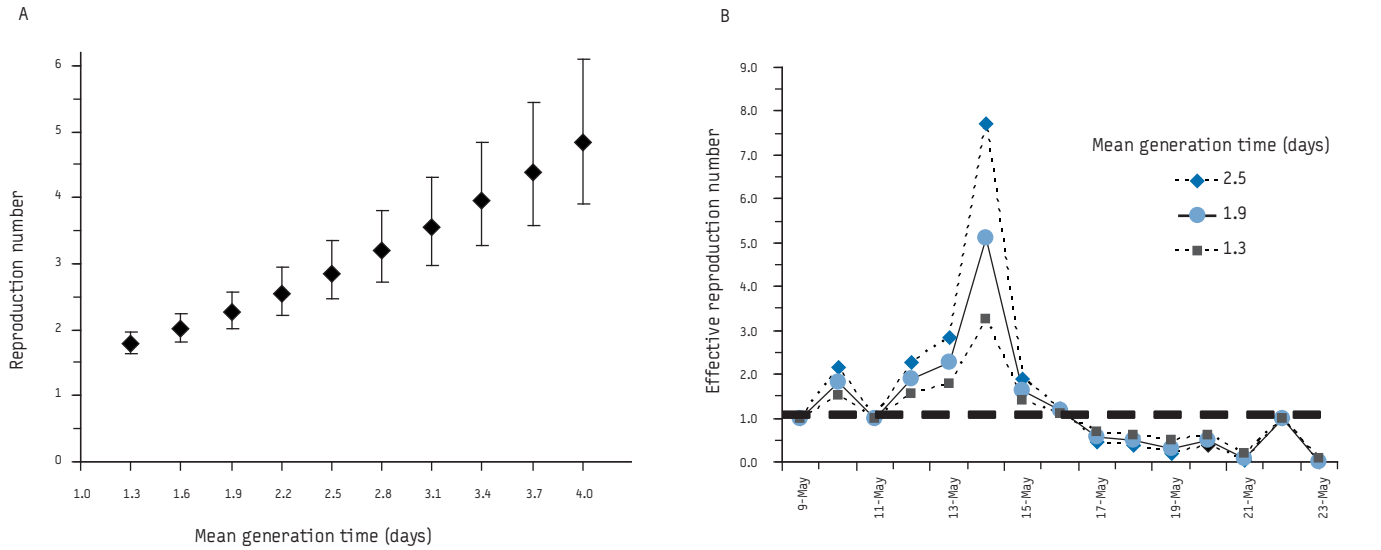
FIGURE 4

Estimates of the reproduction number for the epidemic of new influenza A(H1N1) virus infection in Japan
A) Estimated reproduction number R, based on the initial growth phase of the epidemic (i.e. first eight days)
B) Effective reproduction number R_t, as a function of time



Note:
A) Mean and variance of the generation time were 1.9 days and 0.8 days^2 (given a coefficient of variation of 47%), and the sensitivity of R to different mean generation times is examined. Coefficient of variation is kept constant when the mean generation time is varied.
B) $R_t > 1$ indicates growth of cases at a given point of time, while $R_t < 1$ indicates that the epidemic is in declining trend and may be under control. The horizontal dashed line represents the threshold value, $R_t = 1$. It should be noted that the dates of onset in Japan have yet to be refined, and the precision of R_t estimate may have been influenced by possible delay in diagnosis and reporting

Epidemiology of new influenza A (H1N1) virus infection, United Kingdom, April – June 2009

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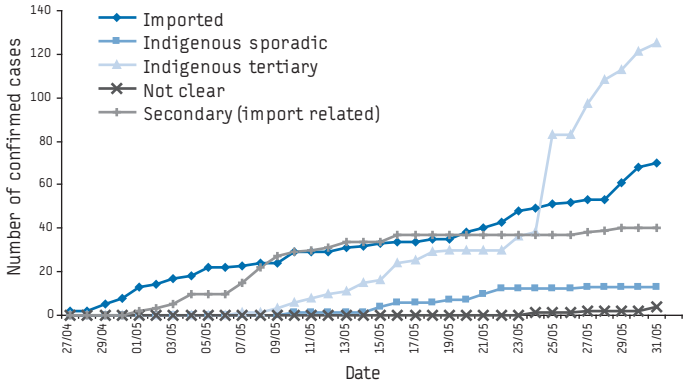
Following the previous report to Eurosurveillance on 14 May 2009, the number of confirmed cases of new influenza A(H1N1) has continued to increase in the United Kingdom. By 31 May, UK surveillance activities had detected a total of 252 confirmed cases. Seventy (28%) were related to travel to the United States and Mexico. There is evidence of spread in households, schools and the community with increases in secondary (n=40), tertiary (n=125) and sporadic (n=13) cases. The new influenza A(H1N1) virus infection continues to cause a mild illness predominately affecting younger age-groups with a low rate of hospitalisation.

Since the identification in late April of cases of acute respiratory infection due to a new influenza A (H1N1) virus in the United States and Mexico [1], the same strain has been detected in an increasing number of countries. By 31 May, the World Health Organization (WHO) had reported 15,510 cases in 53 countries.

The first two confirmed cases of new influenza A(H1N1) virus infection in the United Kingdom (UK) were reported in travellers returning from Mexico to Scotland. The UK response and preliminary epidemiological findings have previously been described [2]. This article provides an update to that report.

Figure 1

Cumulative number of laboratory-confirmed new influenza A(H1N1) cases by day of report and travel history, United Kingdom, 31 May 2009 (n=252)



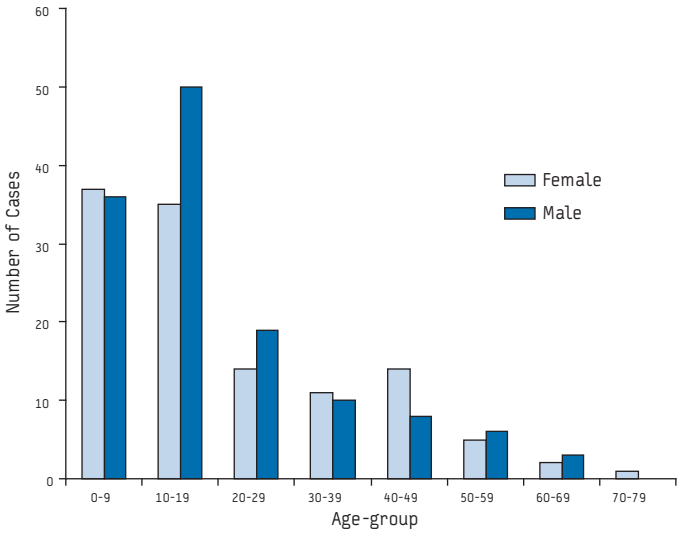
During the period from 27 April to 31 May, a total of 252 confirmed cases have been detected (Figure 1). Initially cases were reported amongst travellers returning from Mexico, and then from the United States. The first indigenously acquired infections in the UK were reported on 1 May and since then the proportion and number of indigenously acquired cases has steadily increased.

Of the 252 confirmed cases, 118 (47%) are female (Figure 2). Cases range in age from 0 to 73 years, with a mean age of 20 years and median age of 12 years.

Of the 252 cases, 28 reported a history of travel in the seven days before disease onset to Mexico and 42 to the United States. Of the remaining 182, 178 cases reported no recent overseas travel and acquired their infection within the United Kingdom. Of these

Figure 2

Cases of laboratory confirmed new influenza A(H1N1) by age-group and sex, United Kingdom, 31 May 2009 (n=251*)

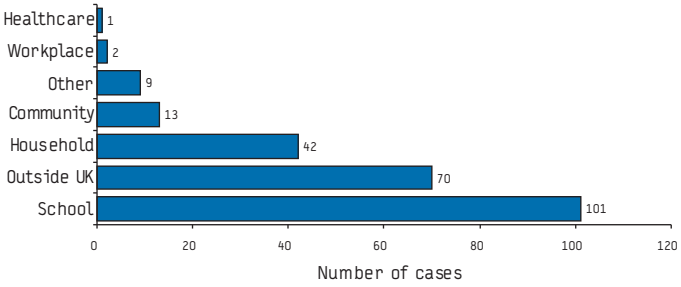


*Age missing for one case.

178 indigenous cases, 40 were secondary (contact within seven days of onset with a travel-associated case); 125 were tertiary (contact within seven days of onset with a secondary case) and 13 sporadic (no travel or contact with a confirmed case in the seven days before onset). Follow-up is still underway for four cases. Amongst the indigenous cases, infection has been linked to likely transmission in a school setting for 101 cases, a household setting for 42 cases, workplace for two cases and health care setting for one case (Figure 3).

FIGURE 3

Setting/source of acquisition of new influenza A(H1N1) virus infection, United Kingdom, 31 May 2009 (n=238*)



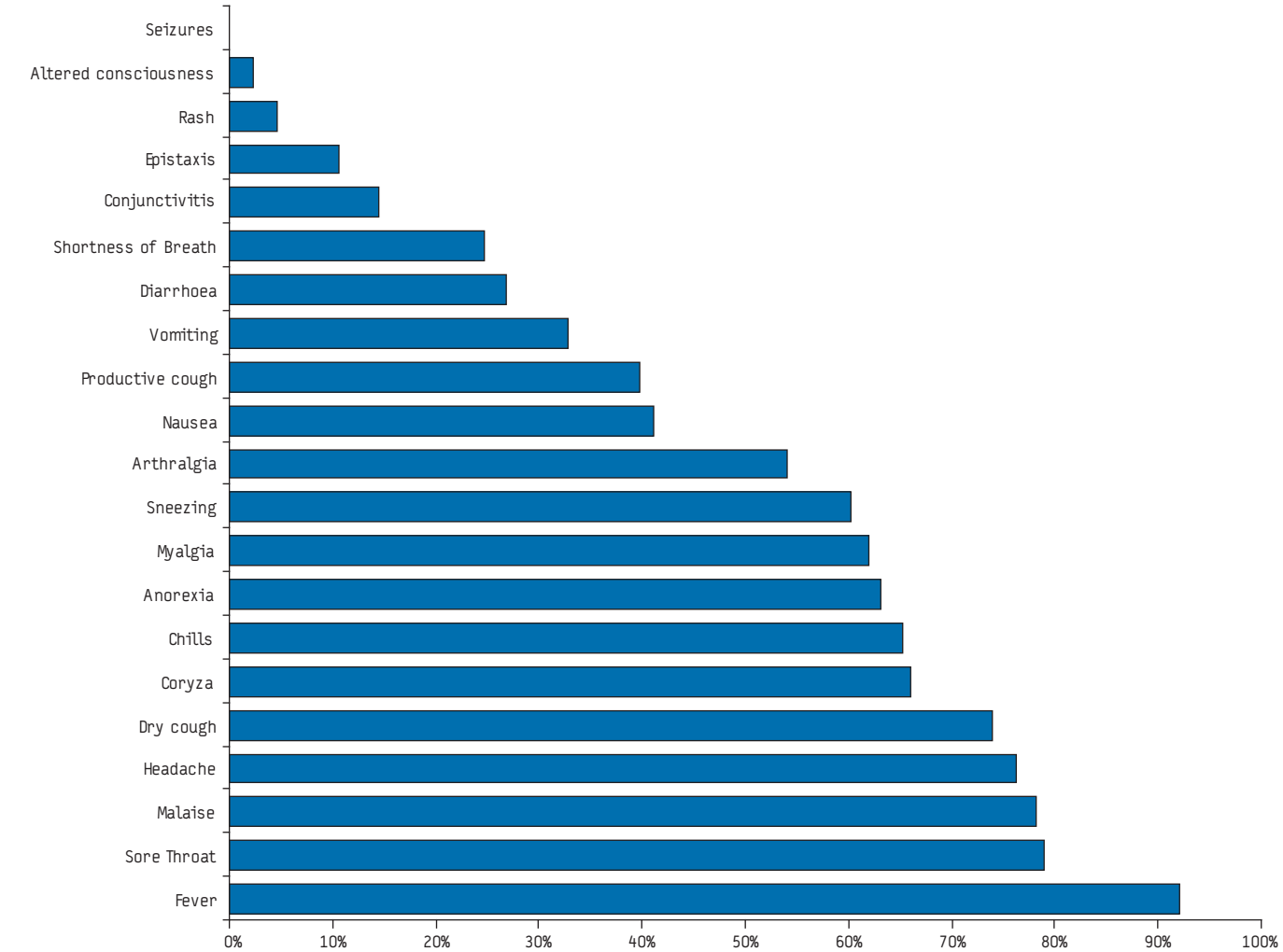
* Investigation is still underway for 14 cases.

The First Few Hundred (FF100) project aims to collect information about a limited number of the earliest laboratory-confirmed cases of new influenza A(H1N1) and their close contacts [3] to gain an early understanding of some of the key clinical, epidemiological, and virological parameters of this infection and to facilitate real time modelling efforts. By 31 May, 175 confirmed cases had been entered into the FF-100 database. Clinical information gathered on these cases shows they continue to present with symptoms typical for influenza (Figure 4).

Up to 31 May, four cases have been hospitalised for clinical reasons. No UK case is known to have died.

FIGURE 4

Clinical presentation of confirmed cases of new influenza A(H1N1) virus infection, United Kingdom, 31 May 2009 (n=175)



HPA and the Health Protection organisations for Scotland, Wales and Northern Ireland have a number of enhanced influenza surveillance systems that are currently operational [4] and that provide an indication of influenza activity in the general population:

- A number of general practitioner (GP) sentinel schemes that collect information on patient consultation rates with influenza-like illness;
- National Health Service (NHS) direct and NHS-24 telephony systems which monitor call rates for colds/flu in the community;
- GP sentinel virological surveillance schemes to monitor circulating respiratory viruses in the community;
- Mortality surveillance based on routine death registration data.

To date, there have not been significant signals of increased influenza activity through these systems, which have established thresholds for widespread circulation of influenza. Outputs from these systems are published on a daily and weekly basis on the HPA website [5]. Further work is on-going to describe more fully the emerging epidemiological, virological and clinical characteristics of this novel influenza virus including in-depth field investigations of individual cluster events in settings such as schools.

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Evaluation of four real-time PCR assays for detection of influenza A(H1N1)v viruses

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The sensitivity and specificity of four real-time PCR assays (HPA A(H1)v, CDC A (H1)v, HPA A(N1)v and NVRL S-OIV assays) were evaluated for detection of influenza A(H1N1)v viruses. Nose and throat swab samples containing influenza A(H1N1)v viruses, seasonal influenza AH3N2, AH1N1, influenza B viruses, or negative for influenza viruses were tested by the four assays. Specificity was also analysed using influenza A viruses of different subtypes and non-related respiratory viruses. The sensitivities and specificities of the four assays were in a similar range and suitable for diagnostic use. The HPA (H1)v and the S-OIV assays were the most sensitive assays for use as a first line test, but the S-OIV assay was less specific, detecting all avian subtypes of influenza A viruses tested. The results of this study demonstrate that the concurrent use of primary diagnostic and confirmatory assays provides rapid and accurate assessment of confirmed cases, and allows appropriate management of patients.

Introduction

The recent emergence of new influenza A(H1N1) virus (henceforth: influenza A(H1N1)v virus, where v stands for variant, according to nomenclature agreed by the World Health Organization Global Influenza Surveillance Network – WHO GISN) in humans [1-2] has led to the requirement for sensitive and specific assays for the differential diagnosis and confirmation of influenza A(H1N1)v virus infections, necessary to guide public health actions. Real-time PCR is widely considered the gold standard for molecular detection of influenza viruses due to its high assay specificity, sensitivity and broad linear dynamic range. In the present study, the performance (including sensitivity and specificity) of four real-time PCR assays designed to detect influenza A(H1N1)v viruses in respiratory specimens has been evaluated. Two assays are based on detection of haemagglutinin (HA), one on the detection of neuraminidase (NA) and one on the matrix (M) gene.

HPA (H1)v assay

The influenza A(H1)v specific assay of the Health Protection Agency (HPA) contains primers and a dual-labelled TaqMan MGB probe (Applied Biosystems) targeting conserved sequences in the HA gene of A(H1N1)v viruses, and the positive control swine A(H1N1) virus A/Aragon/3218/2009, in a 1-step TaqMan PCR assay [3]. The advantage of using a genetically distinct positive control virus (A/Aragon/3218/2008) is that false positives can be differentiated by sequence from true positives.

CDC (H1)v assay

The Centers for Disease Control and Prevention (CDC) real-time RT-PCR kit designed for the detection and characterisation of influenza A(H1N1)v viruses contains a panel of oligonucleotide primers and dual-labelled hydrolysis probes [4]. The CDC (H1)v primer and probe set evaluated in this study has been designed to specifically detect A(H1)v influenza in a one-step RT-PCR assay.

HPA (N1)v assay

The influenza A(N1)v real-time assay (HPA) is a two-step TaqMan PCR assay incorporating oligonucleotide primers and a dual-labelled MGB TaqMan probe for the detection of the NA gene of influenza A(H1N1)v viruses and the positive control virus A/Aragon/3218/2008 [5]. The assay has been designed to be performed in conjunction with the influenza A(H1)v specific assay, to provide confirmation of diagnosis of influenza A(H1N1)v virus infection.

S-OIV assay

The swine-origin influenza virus (S-OIV) assay (National Virus Reference Laboratory, NVRL, Dublin) is a real-time one-step RT-PCR assay containing primers and a dual-labelled hydrolysis probe targeting the M gene of influenza A viruses other than seasonal A(H1N1) and A(H3N2) viruses [6].

Methods

Respiratory samples (85 nose or throat swabs) were submitted as part of the influenza A(H1N1)v virus investigation in the United Kingdom. Of these, 43 influenza A-positive, untypable, M gene sequence-confirmed cases of influenza A(H1N1)v, and 42 A(H1N1) v-negative samples containing seasonal influenza A(H1N1), A(H3N2) or influenza B, or negative for influenza viruses, were analysed using the real-time assays. In addition, specificity was evaluated using representative influenza A viruses of HA subtype H5, H6, H7 and H9, and a panel of non-related respiratory viruses: respiratory syncytial viruses (RSV A and RSV B), parainfluenza viruses, rhinoviruses, human metapneumoviruses (hMPV) and corona viruses. Viral RNA was purified from clinical samples and viral cultures using the Biomerieux NucliSens easyMAG system.

Specimens were tested according to the protocol provided for each assay. All assays were run on an ABI Taqman 7500 Fast Thermal Cycler in standard (one-step assays) or Fast (two-step) mode. All samples were tested in duplicate. Discrepant results were confirmed by repeat testing. Ct values of <40.00 were considered to be positive for detection of viral RNA.

Results

The relative sensitivity of the assays was compared by analysing a 10-fold dilution series of A/England/195/2009(H1N1)v (nose swab sample).

No cross-reaction was observed when the four real-time assays were used to test 22 seasonal influenza viruses, or other respiratory viruses. A panel of representative influenza A viruses of different subtypes was also analysed (Table 2).

The HPA (H1)v and CDC (H1)v specific assays showed no cross-reactivity with any of the other influenza A subtypes analysed. The HPA (N1)v confirmatory assay detected one influenza A(H5N1) virus, but showed no cross-reactivity with other subtype viruses. The S-OIV assay showed cross-reactivity with all of the influenza A viruses analysed.

When 43 true positive samples were analysed, 36 were positive in all four real-time PCR assays (Table 3).

Four false negative and two equivocal results were observed with the CDC (H1)v assay. One equivocal result was observed with the S-OIV assay. Two samples were negative with either the HPA (H1)v or (N1)v assays, but when these assays were performed in parallel, as recommended, one false negative result was observed. No false-positives were detected in the 42 influenza A(H1N1)v virus-negative samples with any of the four real-time assays.

The Ct values obtained by analyses with the real-time assays of the 43 confirmed influenza A (H1N1)v virus samples are shown in Figures 1a-c. A total of 42 true negative and 43 true positive samples were tested in all assays. Comparison of the HPA (H1)v and CDC (H1)v assays showed that of the 43 true positives tested, 41 were detected in the HPA (H1)v assay (Figure 1a). Thirty seven were positive and 2 equivocal in the CDC (H1)v assay. Three samples positive in HPA (H1)v assay were negative in the CDC assay and 1 sample positive in the HPA (H1)v assay was equivocal in the CDC (H1)v assay.

Of the 43 true positives, 41 were positive in the HPA (H1)v assay and 42 in the S-OIV assay (Figure 1b). One sample gave an equivocal result with the S-OIV assay.

Comparison of the HPA (H1)v diagnostic assay with the HPA (N1)v confirmatory assay demonstrated that the two assays correlate well, with a correlation coefficient of r = 0.97 (Figure 1c).

The precision of the HPA (H1)v and (N1)v real-time assays was assessed by the coefficient of variation (CV) and standard deviation (SD) of the replicate Ct measurements (n=37 and n=9 respectively) for the assay-positive control on diagnostic assay runs. The CV for the mean Ct values obtained with the (H1)v and (N1)v assay-positive controls was 3% and 2% respectively.

Table 1

End-point detection of A/England/195/2009 (H1N1)v by four real-time PCR assays

Dilution A/Eng/195/2009(H1N1)v	Mean Ct values			
	HPA (H1)v	CDC (H1)v	HPA (N1)v	S-OIV
1.00E-03	18.35	24.85	23.50	21.85
1.00E-04	21.35	28.25	27.15	25.30
1.00E-05	24.60	31.75	30.60	28.45
1.00E-06	27.95	35.20	34.25	31.70
1.00E-07	30.65	38.70	36.10	37.60
1.00E-08	32.95	Neg	38.80	36.85
1.00E-09	Neg	Neg	Neg	Neg

Table 2

Specificity of four real-time PCR assays with representative influenza A subtype virus isolates

Influenza A virus subtype	HPA (H1)v	CDC (H1)v	HPA (N1)v	S-OIV
A/Cambodia/R0405050/2007 RG[a] H5N1	Neg	Neg	Neg	28.02
A/Indonesia/6/2005 H5N1	Neg	Neg	31.77	27.27
A/Chicken/Turkiye/Av05/2006 H5N1	Neg	Neg	Neg	25.92
A/Vietnam/1203/2004 H5N1	Neg	Neg	Neg	27.36
A/Duck/Singapore-Q/F119-3/97 H5N3	Neg	Neg	Neg	22.53
A Turkey/England/198/2009 H6N1	Neg	Neg	Neg	30.61
A/AfricanStarling/Q-England/983/79 H7N1	Neg	Neg	Neg	29.06
A/Chicken/Wales/306/2007 H7N2	Neg	Neg	Neg	30.54
A/Quail/HongKong/G1/97 H9N2	Neg	Neg	not done	26.08

[a] derived by reverse genetics

Table 3

Comparison of HPA (H1)v, CDC (H1)v, HPA (N1)v, and S-OIV real-time PCR assays on sequence confirmed swine-lineage samples

Number of samples	HPA (H1)v	CDC (H1)v	HPA (N1)v	S-OIV
36	+	+	+	+
1	+	-	+	Equiv*
2	+	-	+	+
1	-	+	+	+
1	-	-	-	+
1	+	Equiv*	+	+
1	+	Equiv*	-	+
Sensitivity (%)	95.4	90.7	95.4	100

* Weak positive in one replicate

Figure 1a

Comparison of HPA (H1)v and CDC (H1)v assay Ct values

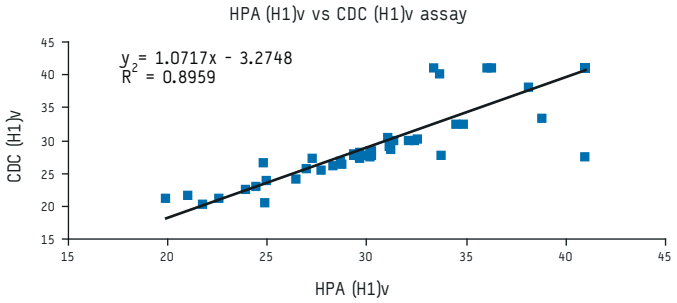


Figure 1b

Comparison HPA (H1)v and S-OIV assay Ct values

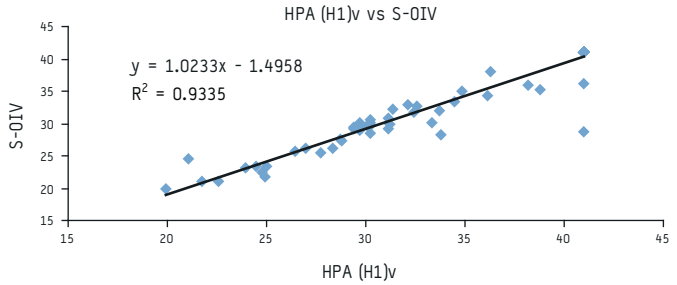
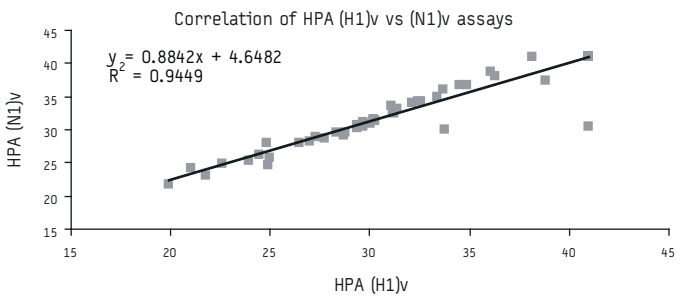


Figure 1c

Correlation of Ct values obtained with HPA (H1)v and (N1)v assays



Conclusions

The sensitivities and specificities of the four assays were in a similar range and suitable for diagnostic use. The HPA (H1)v and the S-OIV assays were the most sensitive assays for use as a first line test, but the S-OIV assay was less specific, detecting all avian subtypes of influenza A viruses tested. For confirmation, an assay in another gene such as the HPA (N1)v could be employed. The results obtained with the HPA (H1)v and (N1)v assays correlated well and, in addition, intra-assay variability of the HPA (H1)v and (N1)v assays was shown to be acceptable with values for the coefficient of variation (CV) <5%.

Because the security of a diagnostic result for influenza A(H1N1)v virus is important for public health actions, the use of primary detection and confirmatory assays as described here is appropriate. The use of the HPA (H1)v and (N1)v assays together provides rapid and accurate assessment of confirmed cases, and enables appropriate management of patients.

Acknowledgements

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*Authors' correction:

The name of the third author was corrected on 13 June 2012 at the request of the authors.

Origins of the new influenza A(H1N1) virus: time to take action

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To gain insight into the possible origins of the 2009 outbreak of new influenza A(H1N1), we performed two independent analyses of genetic evolution of the new influenza A(H1N1) virus. Firstly, protein homology analyses of more than 400 sequences revealed that this virus most likely evolved from recent swine viruses. Secondly, phylogenetic analyses of 5,214 protein sequences of influenza A(H1N1) viruses (avian, swine and human) circulating in North America for the last two decades (from 1989 to 2009) indicated that the new influenza A(H1N1) virus possesses a distinctive evolutionary trait (genetic distinctness). This appears to be a particular characteristic in pig-human interspecies transmission of influenza A. Thus these analyses contribute to the evidence of the role of pig populations as "mixing vessels" for influenza A(H1N1) viruses.

Introduction

On 24 April, the World Health Organization (WHO) released the first alert indicating the occurrence of confirmed human cases of swine influenza A(H1N1) in North America [1]. A few days later, the Centers for Disease Control and Prevention in the United States confirmed that these human influenza cases were caused by the same new influenza A(H1N1) virus [2]. Soon after, it was proposed that the current flu outbreak is caused by a new influenza A(H1N1) virus generated from a triple reassortment of human, swine and avian viruses [2-8]. Other publications, including our study presented here, demonstrate that this new influenza A(H1N1) virus most likely evolved from recent swine viruses [9-11].

Methods and results

Protein homology analysis

We used more than 400 protein sequences to analyse the genetic evolution of the new influenza A(H1N1) virus. This set of protein sequences included polymerases PB2, PB1 and PA, hemagglutinin (HA), nucleocapsid (NP), neuraminidase (NA), matrix 1 (MP1), nonstructural 1 (NS1) encoded by the new influenza A(H1N1) virus as well as other homologous proteins from influenza viruses from past flu seasons. Phylogenetic tree topologies revealed that the closest homologies for the new influenza A(H1N1) virus are swine influenza viruses that have been circulating in the United States and Asia for the last decade (Figure 1, Supplementary materials: Figure 1 and Table 1).

Figure 1. Possible origins of the influenza 2009 A(H1N1) virus: a) hemagglutinin and b) neuraminidase proteins
(See Below)

These findings indicate that domestic pigs in North America may have a central role in the generation and maintenance of this virus. This idea is also supported by the observation that protein sequences of the new influenza A(H1N1) virus have close homology to proteins of swine influenza viruses that infected humans in the recent past (Supplementary materials: Figure 1, Figure 2 and Table 2). In fact, a common element of these swine influenza zoonotic transmissions was that humans (mostly swine farm workers) were in direct contact with infected pigs [12-15].

Phylogenetic analysis

To further examine the possible genetic origins of the new influenza A(H1N1) virus, we compared all the available sequences of influenza A(H1N1) viruses circulating in North America for the last two decades (from 1989 to 2009). Protein sequences from avian, swine and human influenza viruses were obtained from the Influenza Virus Resource [16], a database that integrates information gathered from the Influenza Genome Sequencing Project of the National Institute of Allergy and Infectious Diseases (NIAID) and the GenBank of the National Center for Biotechnology Information (NCBI). A total of 5,214 protein sequences were found in this database. After removing identical sequences, a set of 1,699 influenza A proteins including PB2, PB1, PA, HA, NP, NA, MP1, and NS1 proteins were used for analyses of the genetic evolution of influenza A(H1N1) viruses. These analyses provide additional evidence of the role of pig populations as "mixing vessels" for influenza A(H1N1) viruses (Figure 2).

Figure 2. Genetic distinctness of the influenza 2009 A(H1N1) virus: a) hemagglutinin (HA) and b) neuraminidase (NA) proteins; c) phylogenetic trees for PB2, PB1, PA, NP, MP1, and NS1 proteins
(See Below)

Secondly, our analyses also revealed that the new influenza A(H1N1) virus possesses a distinctive evolutionary trait (genetic distinctness), that seems to be characteristic in pig-human interspecies transmission of influenza A (reported cases occurred in Iowa, Maryland and Wisconsin, United States between 1991 and 2006) (Figure 2, Supplementary materials: Figure 2 and Table 3).

Discussion and conclusion

Although limited in sample size, our analyses substantiate the value of molecular screening and phylogenetic assessment for understanding the evolution of influenza viruses and, most importantly, for the early detection of emerging novel viruses that could lead to influenza pandemics. Notably, our analyses revealed

that the new influenza A(H1N1) virus is genetically distinct from other influenza A(H1N1) viruses that have been circulating for the last twenty flu seasons (Figure 2 and Supplementary materials: Figure 2). Influenza viruses with novel antigens (genetic drift) can escape from immune responses induced by prior infection or vaccination and can lead to a pandemic [17].

These observations also reiterate the potential risk of pig populations as the source of the next influenza virus pandemic. Although the role of swine as "mixing vessels" for influenza A(H1N1) viruses was established more than a decade ago [18,19], it appears that the policy makers and scientific community have underestimated it. In fact, in 1998 influenza experts proposed the establishment of surveillance in swine populations as a major part of an integrated early warning system to detect pandemic threats for humans [18,19] but, to some extent, this task was overlooked. For example, a search of influenza sequences in the Influenza Virus Resource [16] revealed that the total number of swine influenza A sequences (as of 19 May 2009) is ten-times smaller than the corresponding number of human and avian influenza A sequences (4,648 compared to 46,911 and 41,142 sequences, respectively). More significantly, in some countries, such as the United States, the national strategy for pandemic influenza [20] assigned the entire preparedness budget (3.8 billion US dollars) for the prevention and control of avian A(H5N1) influenza, overlooking the swine threat [20-22]. In our (the authors') opinion, in this plan, a substantial effort was dedicated to prevent and contain the foreign threat of Asian avian flu, neglecting the influenza threat that the North American swine population presents [23]. Specifically, we believe that the aforementioned strategy ignores the swine farm and industry workers which constitute the population at higher risk of contracting and spreading the hypothetical pandemic influenza virus [24-26].

The current new influenza A(H1N1) outbreak caused by a virus of swine origin represents a new challenge for animal and human health experts. Our institution, the College of Veterinary Medicine at the National Autonomous University of Mexico (Universidad Nacional Autonoma de Mexico, UNAM) is placing a strong emphasis on the establishment of influenza surveillance in swine and avian species to identify novel genetic assortment of the new influenza A(H1N1) and other influenza viruses circulating in Mexico. For example, since 2002, we have been monitoring the genetic evolution of influenza A viruses circulating in Mexican poultry farms [27]. Now, a similar surveillance system will be applied to swine farms. This effort prioritises the use of genetic distinctness as a marker for the detection of novel viruses that could lead to influenza pandemics.

The recent influenza pandemic threat in North America reveals that it is time to take action towards the development of a systemic surveillance system which integrates phylogenetic information of influenza viruses circulating in humans and livestock.

Supplementary materials: Figure 1, Figure 2, Table 1, Table 2, Table 3:
(See Below)

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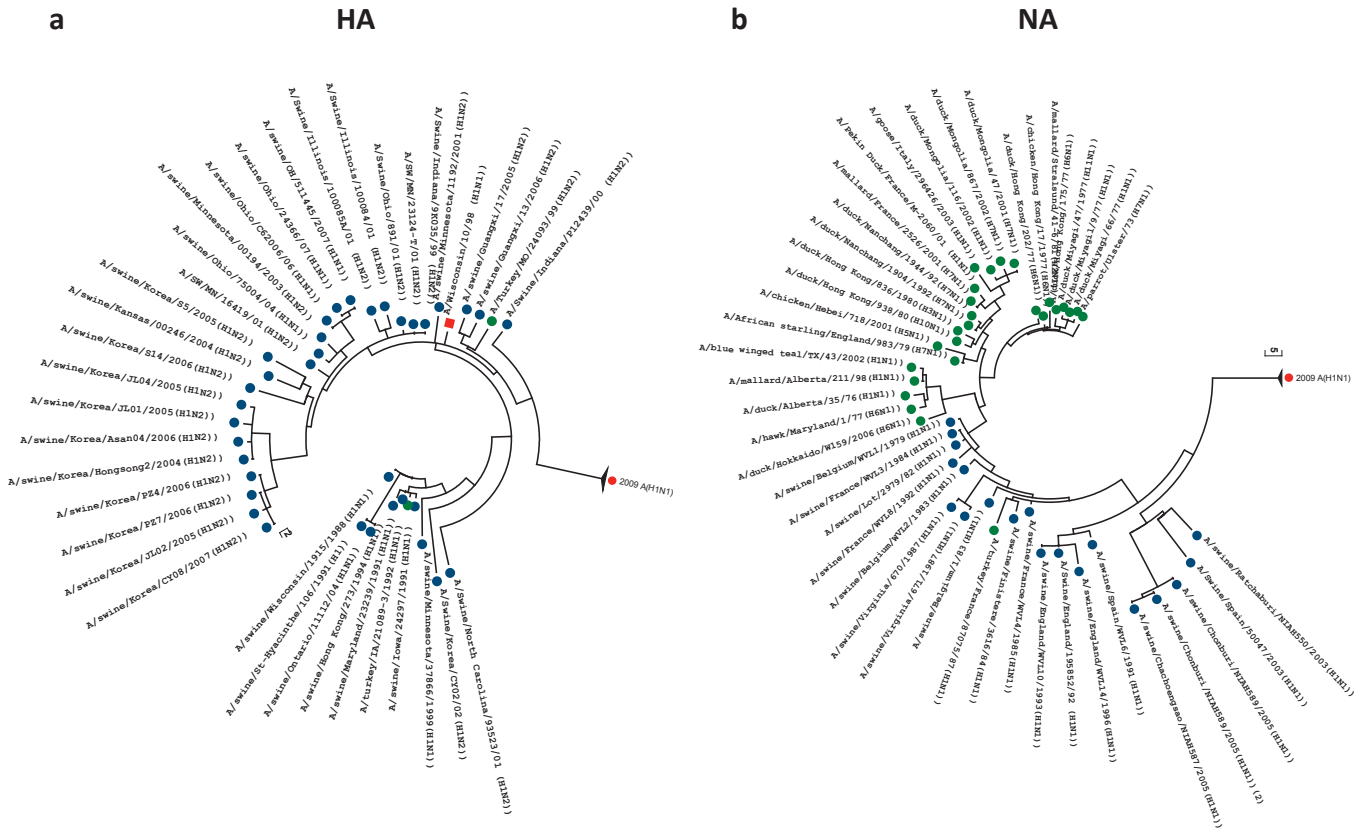
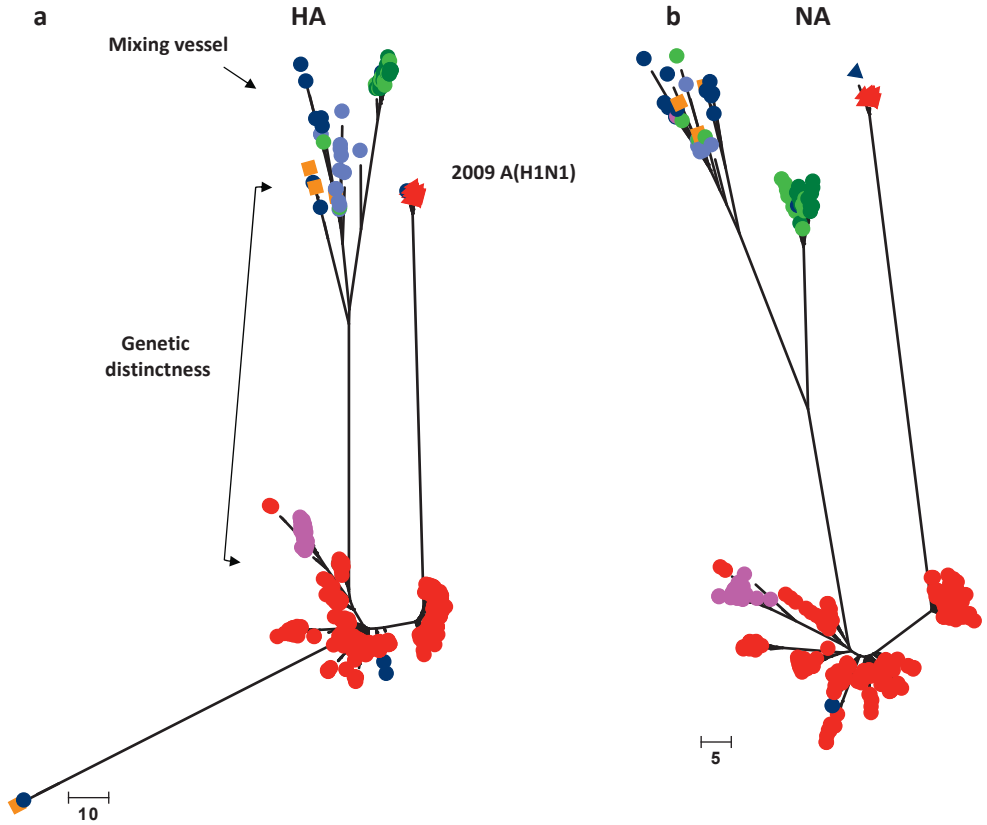
Figure 1. Possible origins of the influenza 2009 A(H1N1) virus: a) hemagglutinin and b) neuraminidase proteins



Protein sequences from the 2009 A(H1N1) virus were retrieved and used for BLAST searches versus the all-species NCBInr protein database. Top-fifty best hits were retrieved from GenBank and used for phylogenetic tree reconstruction using the maximum parsimony method. Phylogenetic trees were rooted using the earliest influenza virus found with the analysis. Proteins from the 2009 A(H1N1) virus (red circles) showed close homology to proteins from swine influenza viruses circulating in Asia, Europe and US (blue circles) and swine influenza viruses that have infected humans in recent past (red squares). Protein relationships with avian influenza virus (green circles) were more distant. Scale bar indicates the number of changes over the whole sequence. Phylogenetic trees for PB2, PB1, PA, NP, MP1, and NS1 proteins, and details of statistical significance of branch order are provided in Supplementary Materials - Figure 1.

Figure 2. Genetic distinctness of the influenza 2009 A(H1N1) virus: a) hemagglutinin (HA) and b) neuraminidase (NA) proteins; c) phylogenetic trees for PB2, PB1, PA, NP, MP1, and NS1 proteins

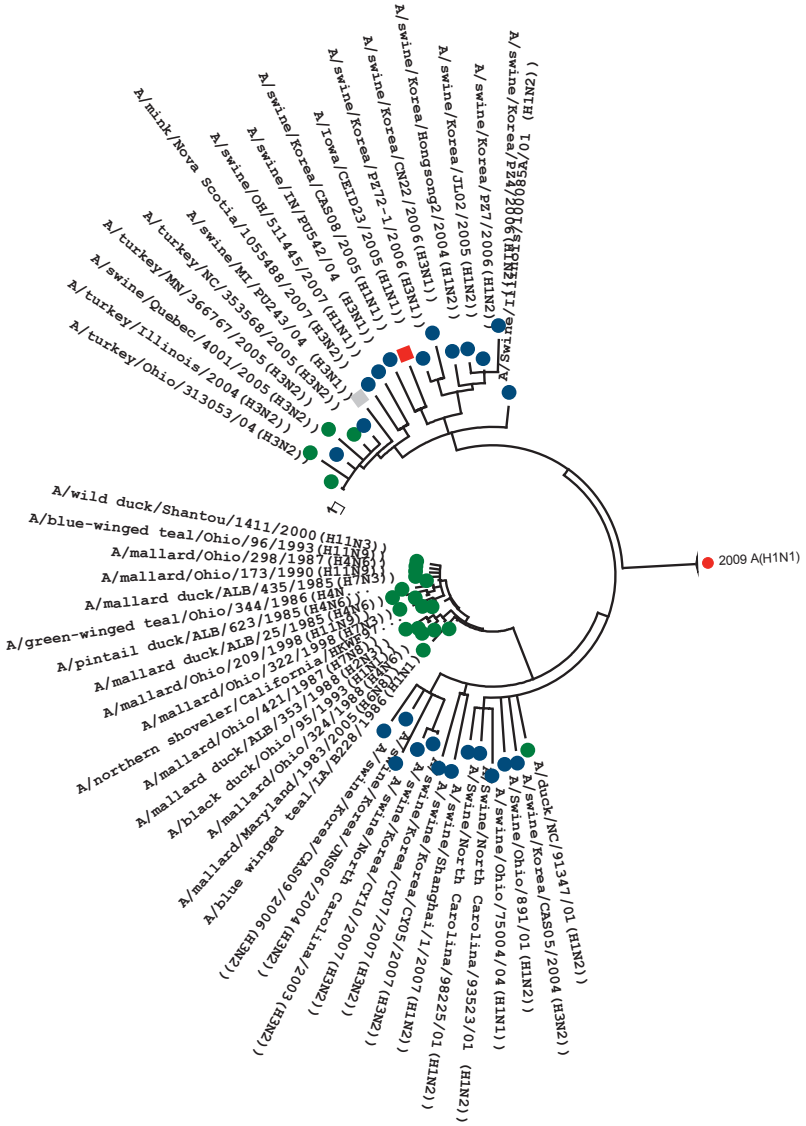


Supplementary materials for

Origins of the new influenza A(H1N1) virus: time to take action

Supplementary Fig. 1. Possible origins of influenza 2009 A(H1N1) virus. **a**, PB2; **b**, PB1 and **c**, PA polymerases; **d**, hemagglutinin; **e**, nucleocapsid protein; **f**, neuraminidase; **g**, matrix protein 1; **h**, nonstructural protein 1. Protein sequences from the 2009 A(H1N1) virus were used for BLAST searches versus the all-species NCBInr protein database. Top fifty best hits were retrieved from GenBank and used for phylogenetic tree reconstruction using the maximum parsimony method. Phylogenetic trees were constructed with the maximum parsimony method using the MEGA software version 4.0 and rooted using the earliest influenza virus isolates obtained with the analyses. The statistical significance of branch order was estimated by the generation of 100 replications of bootstrap resampling of the originally-aligned amino acid sequences. Scale bar indicates the number of changes over the whole sequence.

a **PB2**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A/swine/Korea/PZ7/2006(H1N2))
A/swine/Korea/PZ72-1/2006(H3N1))
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A/mallard/Ohio/173/1990(H11N3))
A/mallard duck/ALB/435/1985(H7N3))
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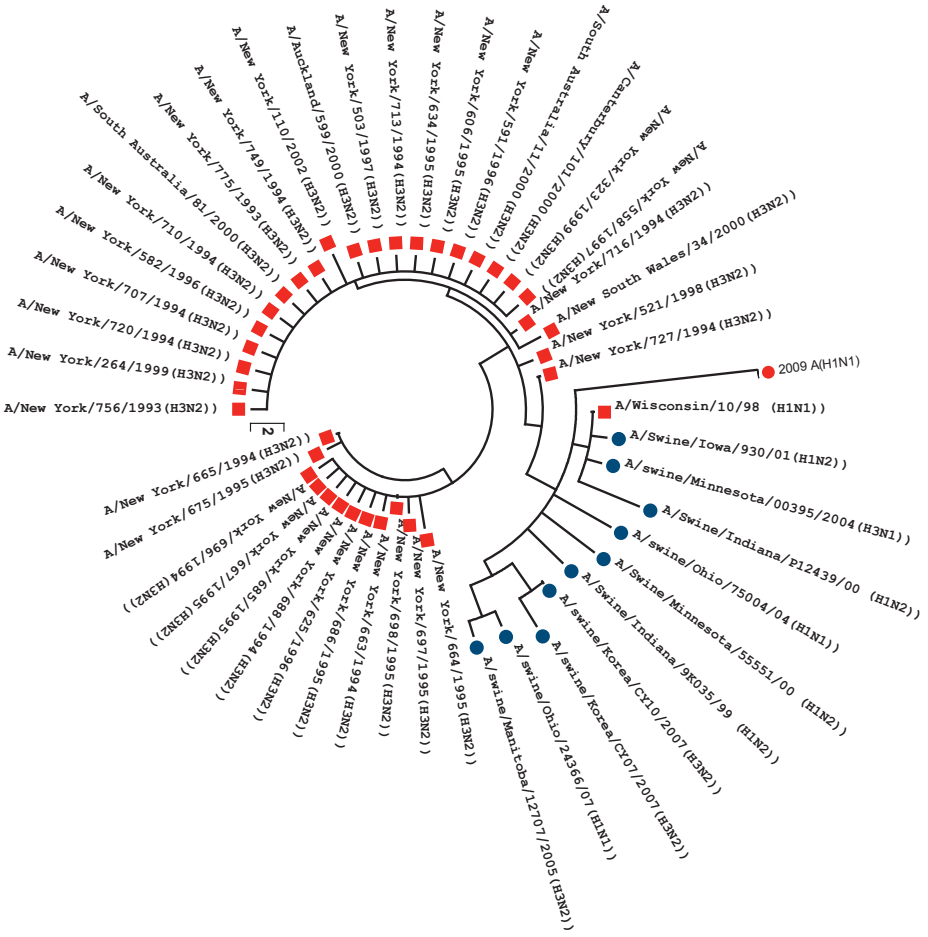
2009 A(H1N1)

b **PB1**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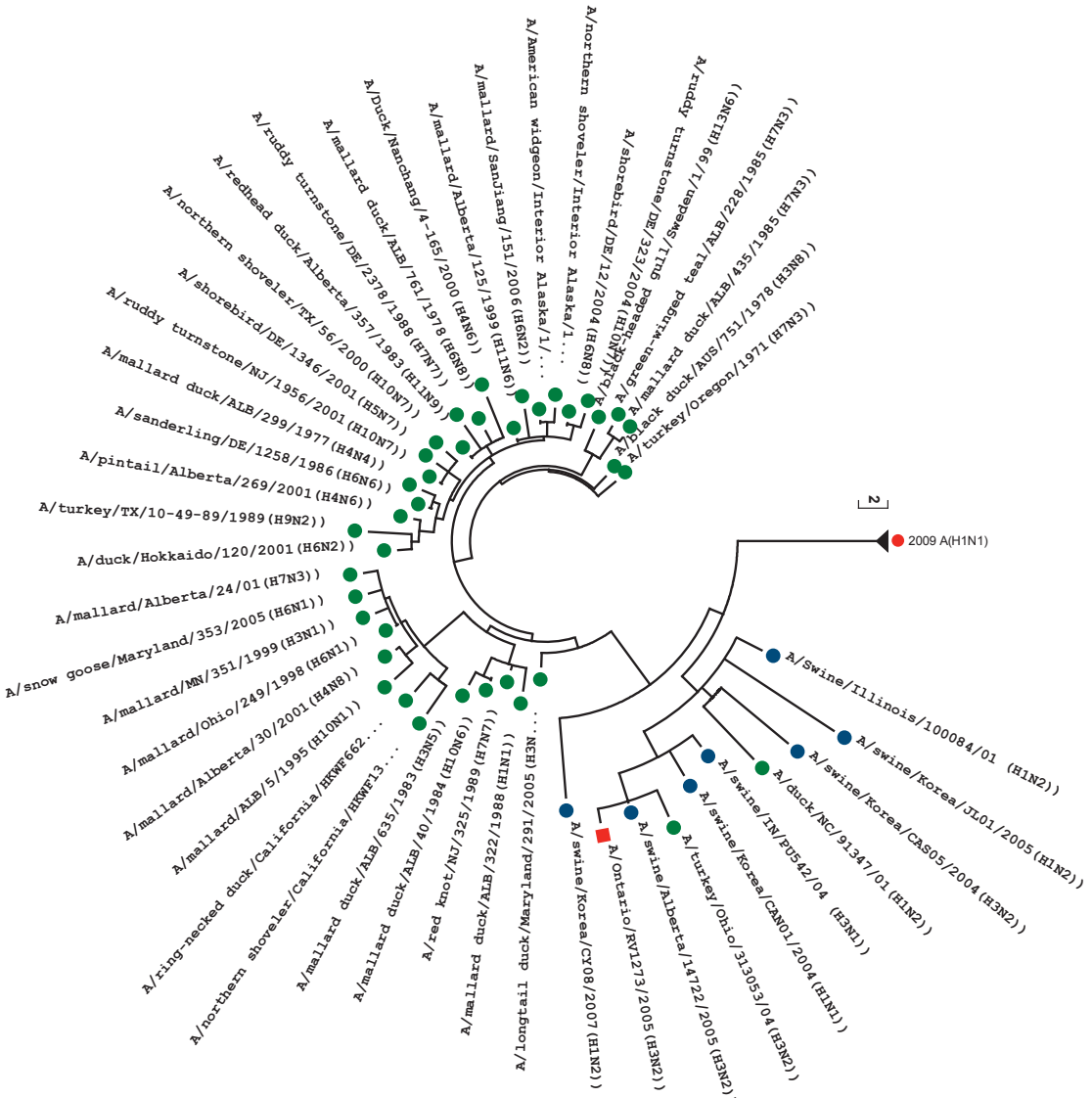
c

PA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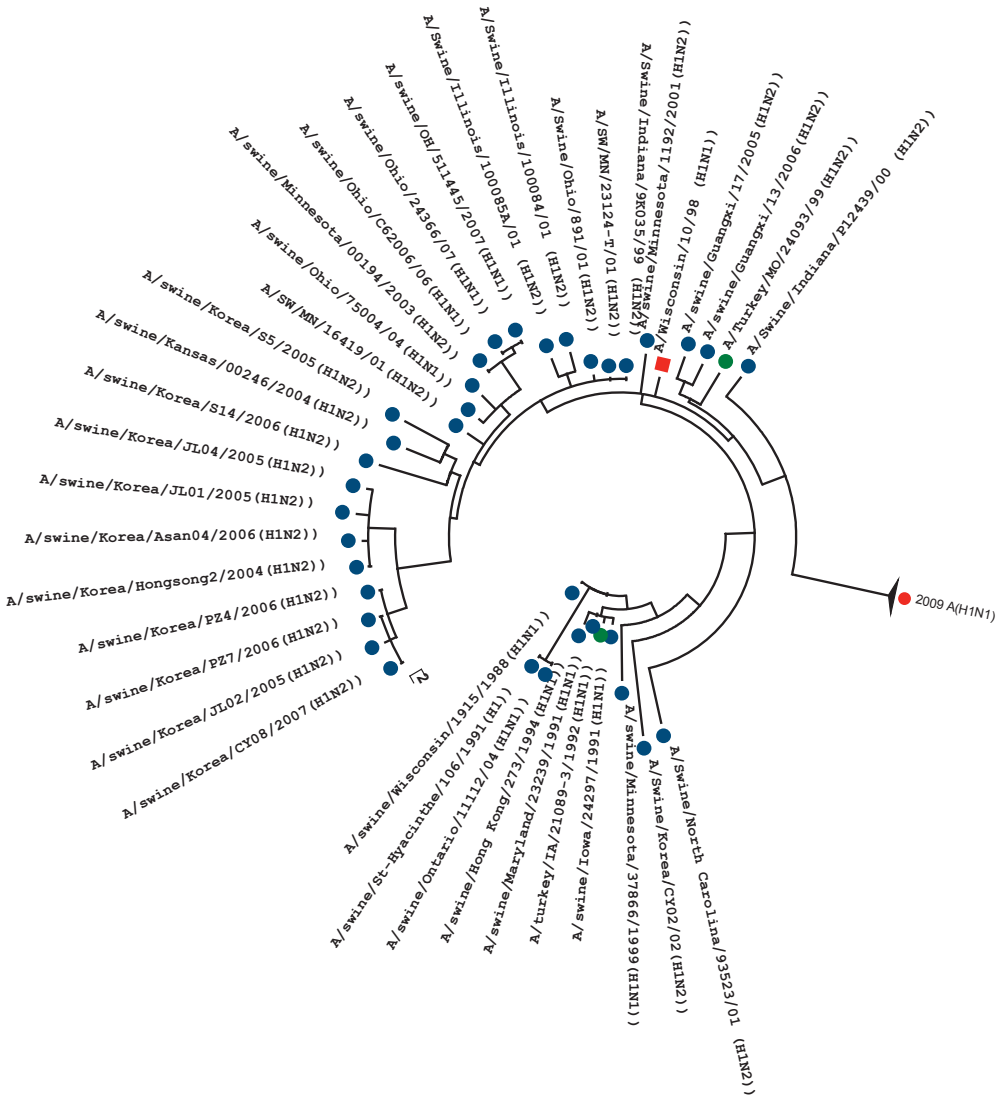
d **HA**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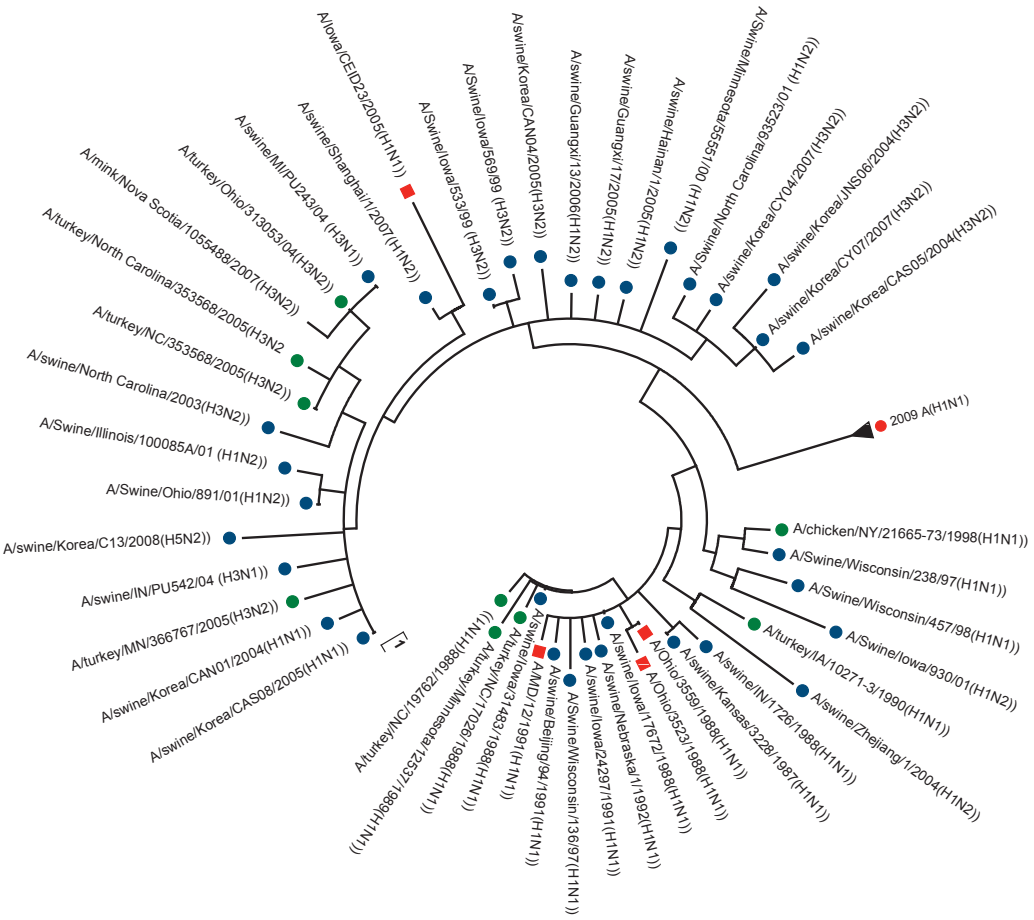
N2

e **NP**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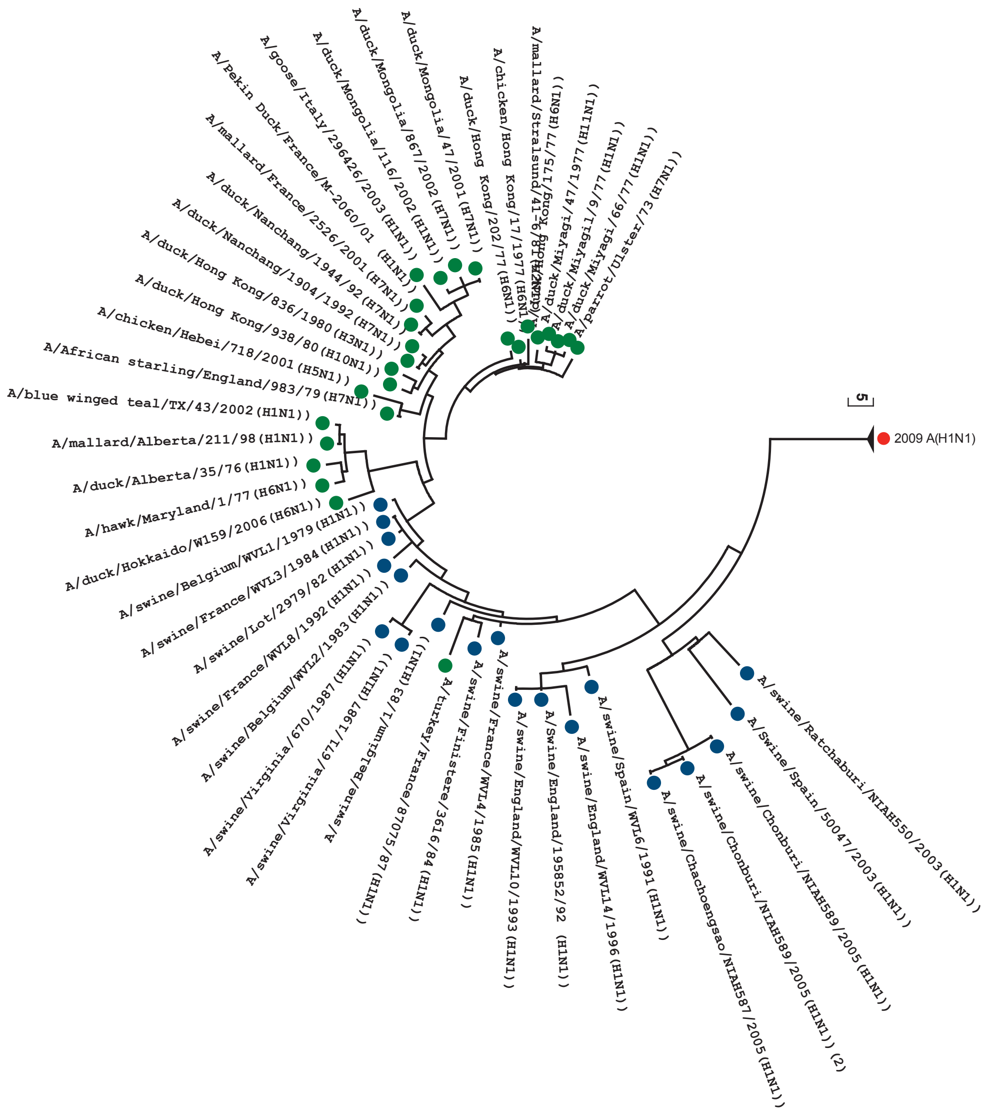
f

NA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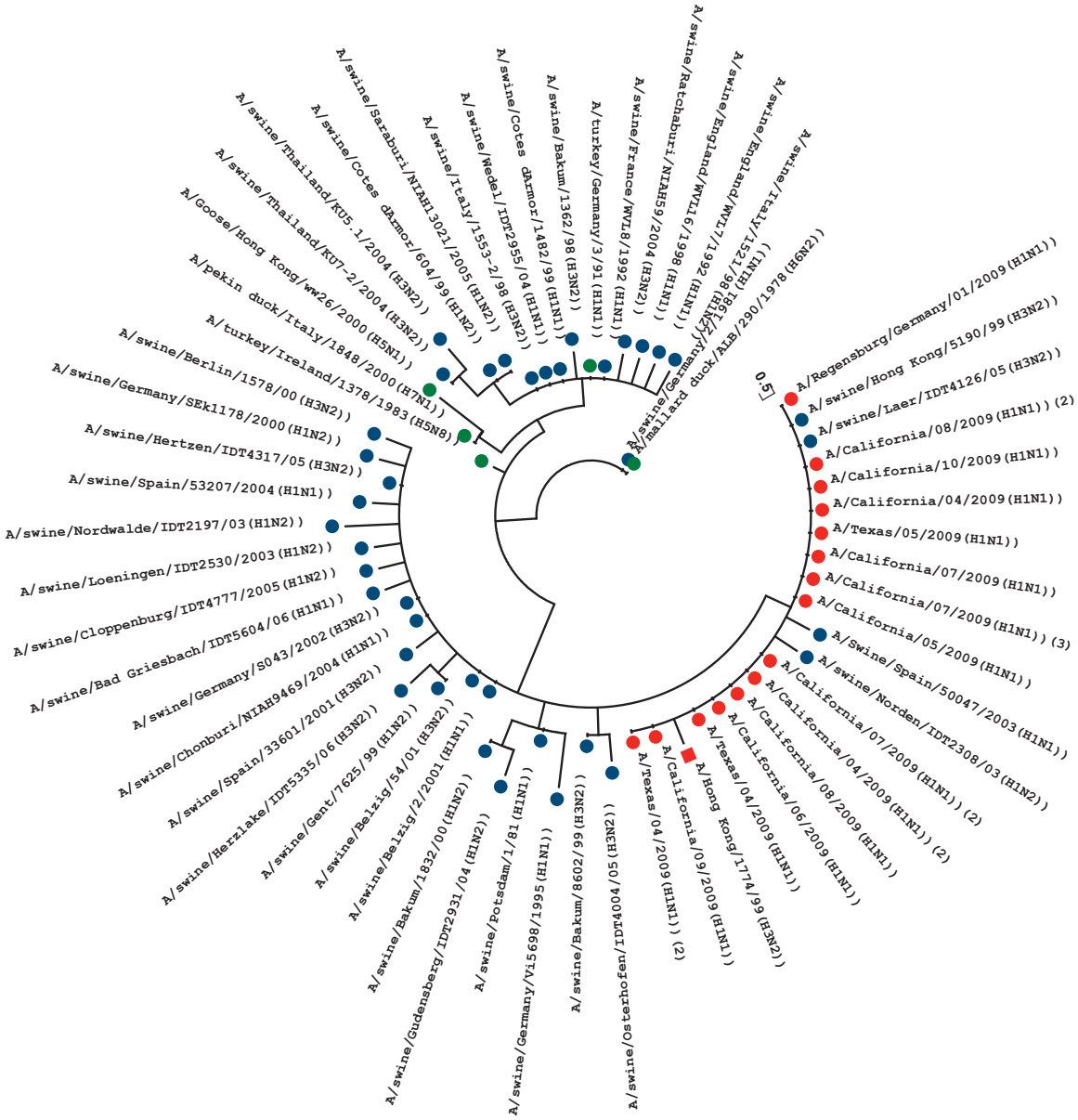
5
2009 A(H1N1)

g

MP1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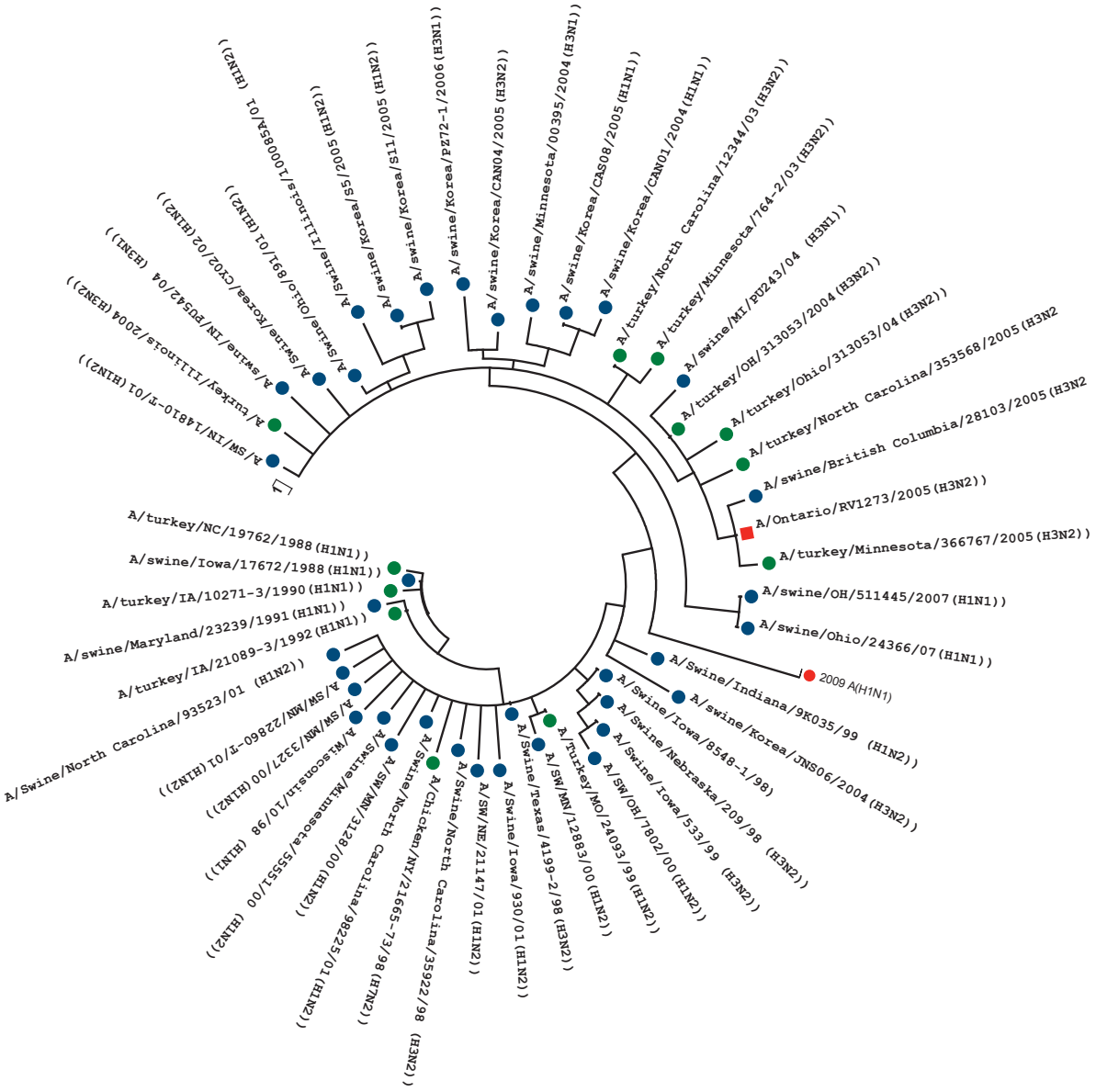
h NS1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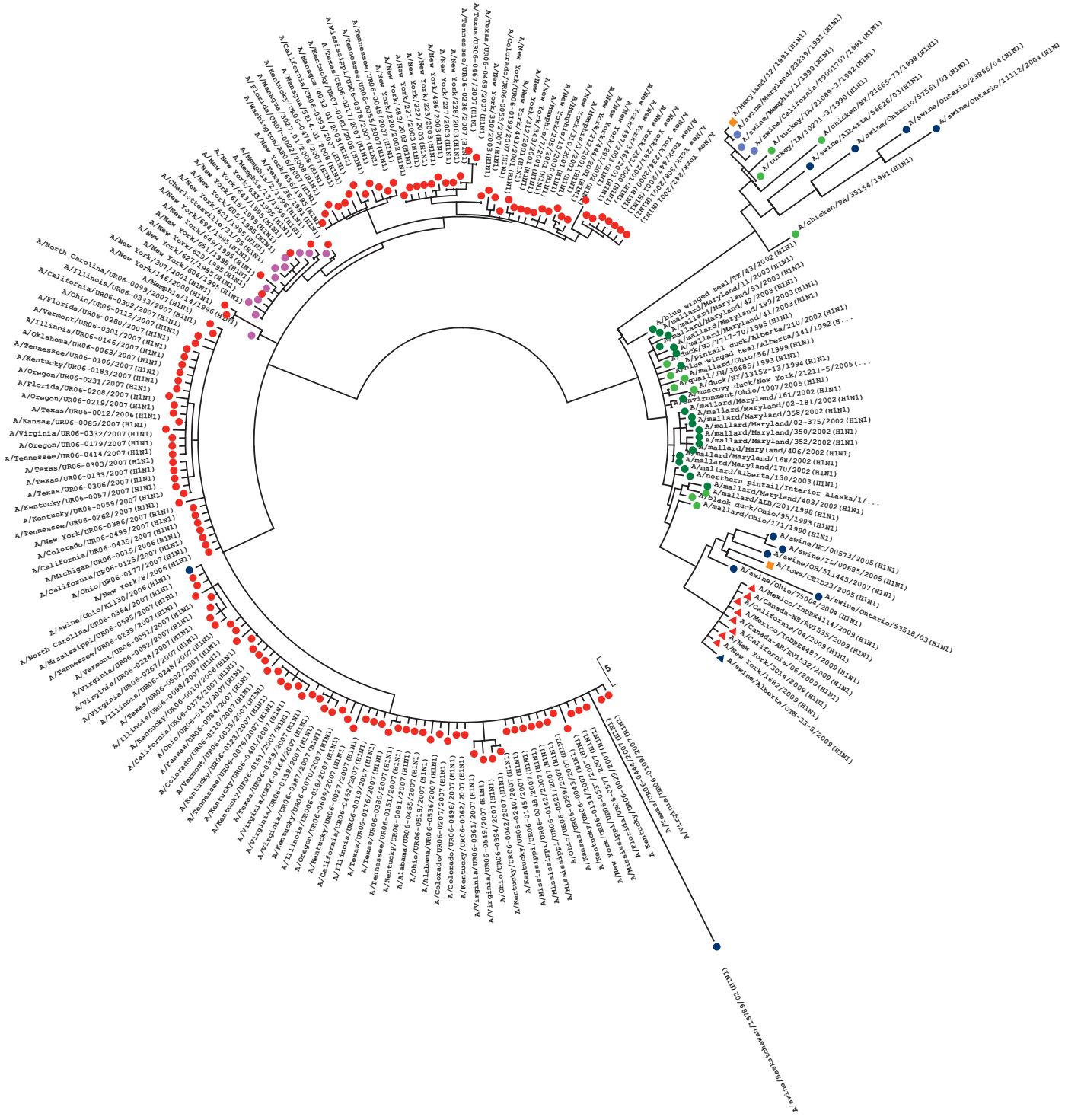
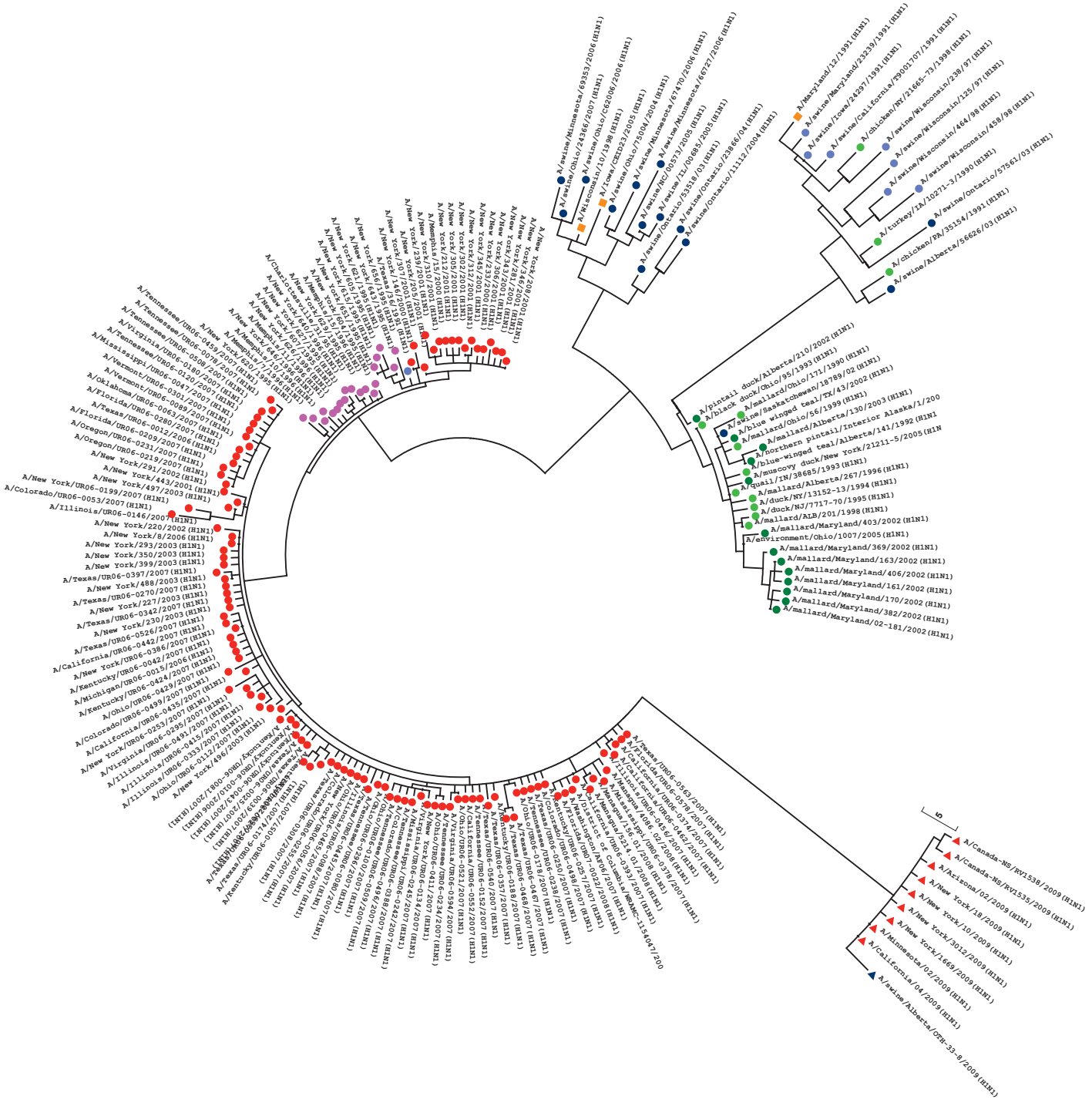
Supplementary Fig. 2. Genetic distinctness of the influenza 2009 A(H1N1) virus. **a,** PB2; **b,** PB1 and **c,** PA polymerases; **d,** hemagglutinin (HA); **e**, nucleocapsid protein (NP); **f,** neuraminidase (NA); **g,** matrix protein 1 (MP1); **h,** nonstructural protein 1 (NS1). Protein sequences from avian, swine and human influenza A (H1N1) viruses circulating in North-America from 1989 to 2009 were retrieved from the Influenza Virus Resource. Sequences were used for unrooted phylogenetic tree construction with the maximum parsimony method. Proteins from the influenza 2009 A(H1N1) virus (red triangles), earlier human (red and pink circles) swine (navy blue and purple circles) and avian (green circles) viruses are shown. Light colors (pink, purple and green) correspond to viruses found between 1989 and 1999 and dark colors (red, navy blue and green) to viruses found between 2000 and 2009. Orange squares represent pig-human interspecies transmission of influenza A cases occurred in Iowa, Maryland and Wisconsin, USA between 1991 and 2006. Scale bar indicates the number of changes over the whole sequence. Phylogenetic trees were constructed with the MEGA software version 4.0. The statistical significance of branch order was estimated by the generation of 100 replications of bootstrap resampling of the originally-aligned amino acid sequences. Scale bar indicates the number of changes over the whole sequence.

a **PB2**



b **PB1**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A/northern pintail/Interior Alaska/1/200
A/mallard/Alberta/141/1992(H1N
A/blue-winged teal/Alberta/141/1992(H1N
A/muscovy duck/New York/21211-5/2005(H1N
A/quail/IN/38685/1993(H1N1)
A/mallard/Alberta/267/1996(H1N1)
A/duck/NY/13152-13/1994(H1N1)
A/duck/NJ/7717-70/1995(H1N1)
A/mallard/ALB/201/1998(H1N1)
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A/Arizona/02/2009(H1N1)
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A/New York/10/2009(H1N1)
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A/New York/1669/2009(H1N1)
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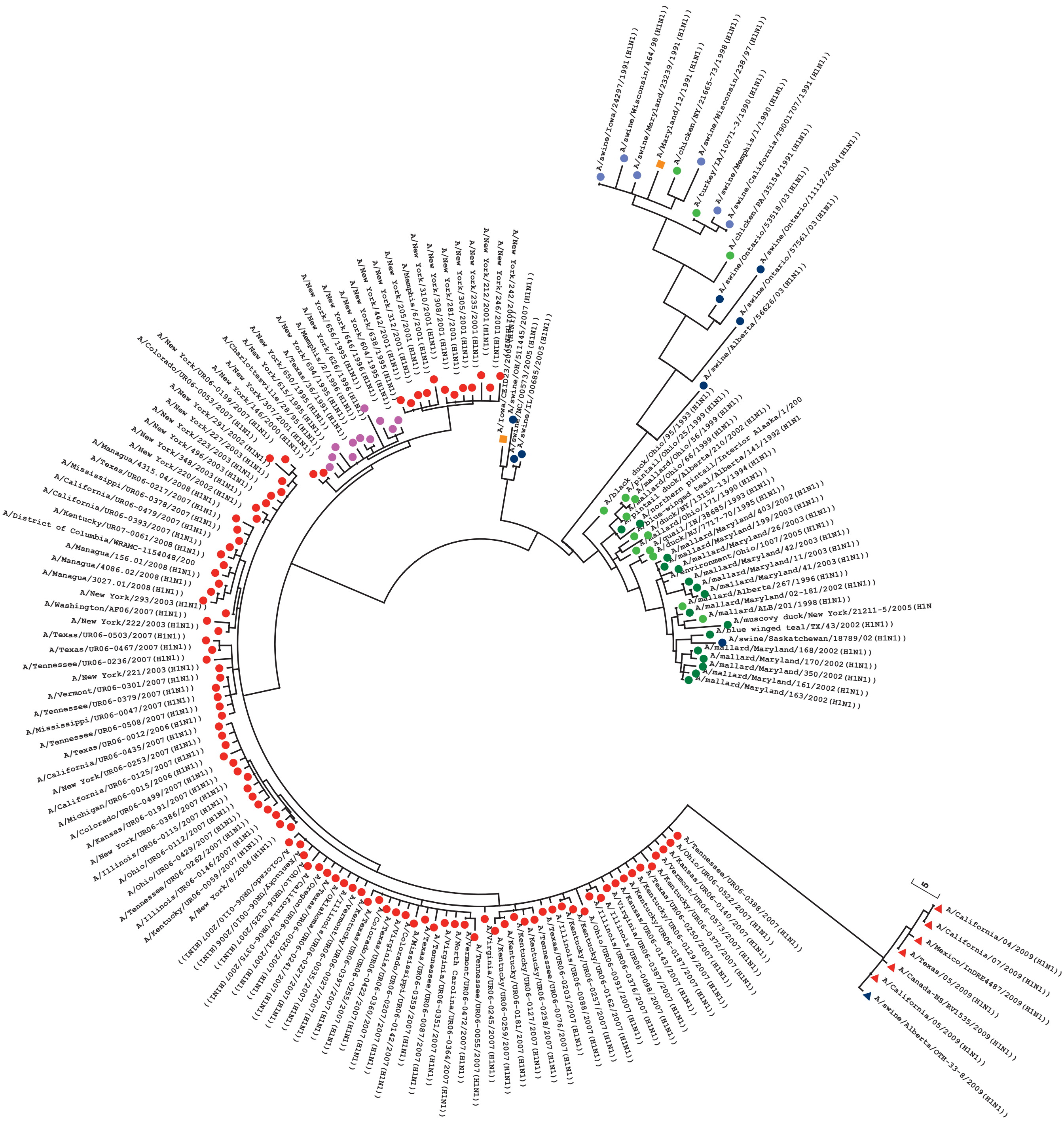
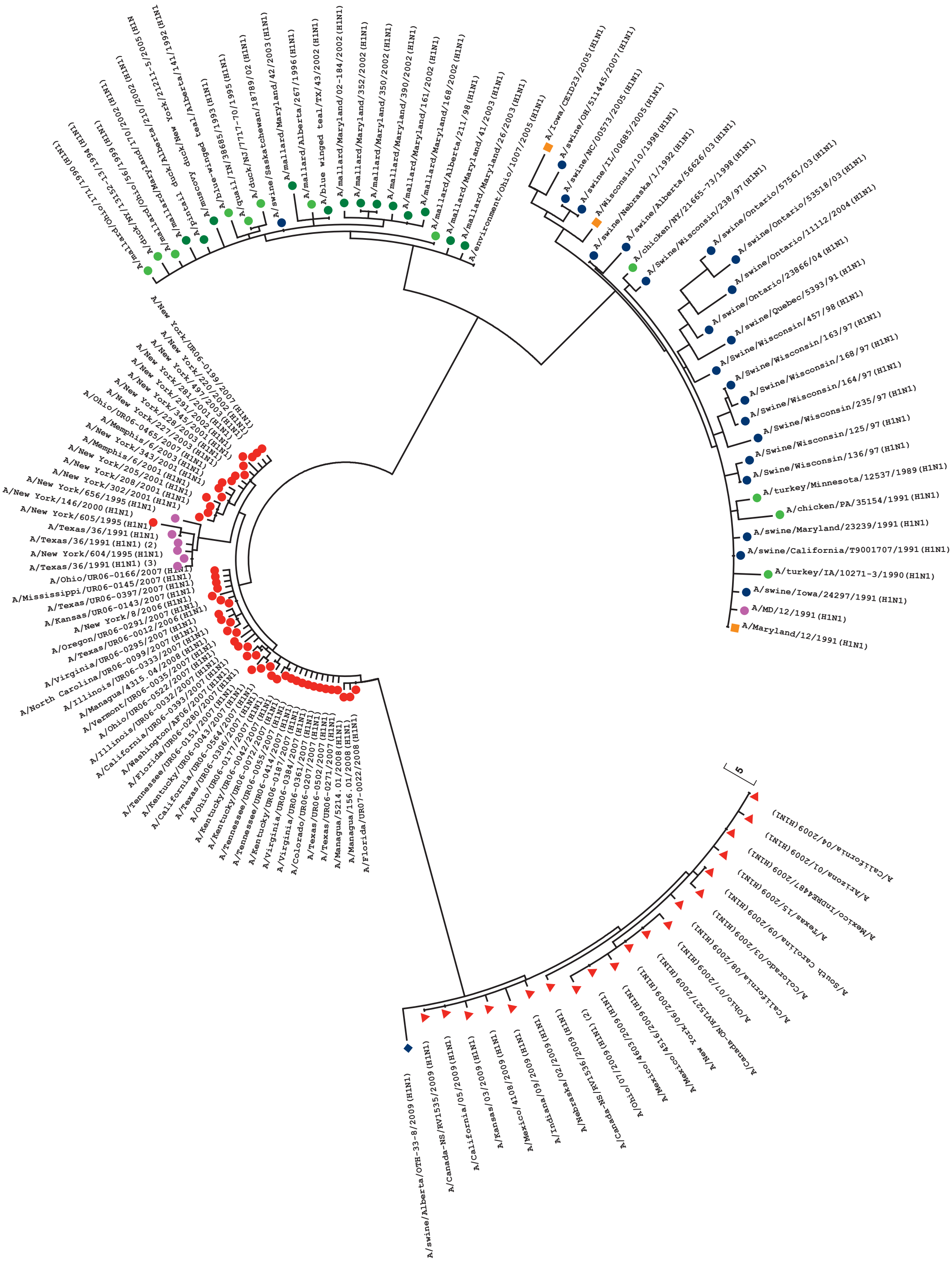
c

PA

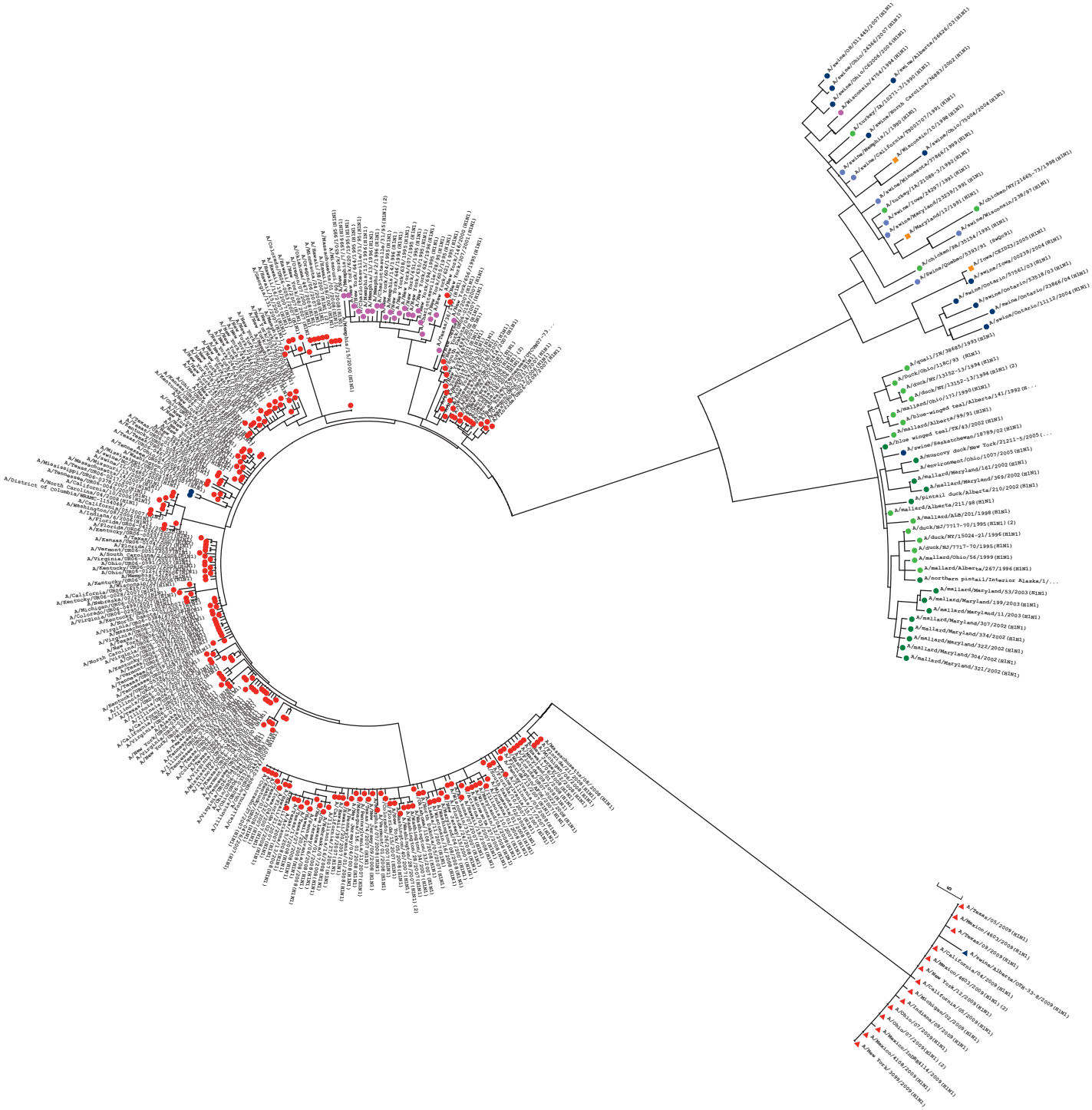



d
HA
10

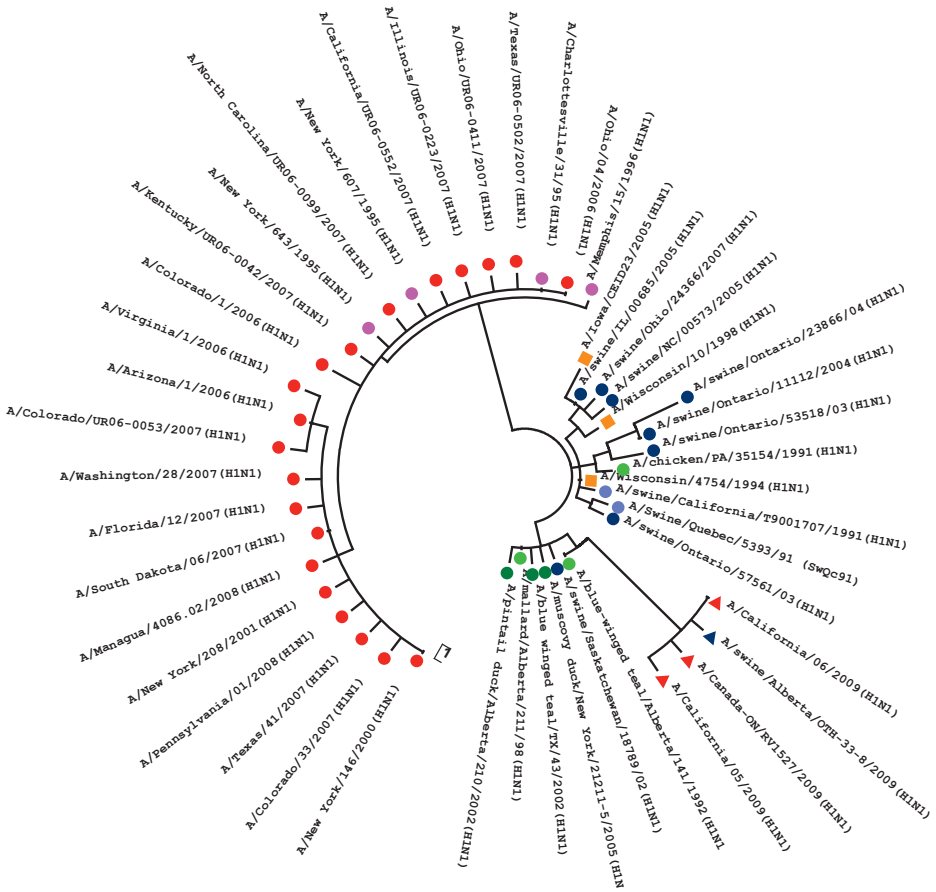
e **NP**



f **NA**



g **MP1**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A/California/UR06-0552/2007 (H1N1)
A/New York/607/1995 (H1N1)
A/North Carolina/UR06-0099/2007 (H1N1)
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A/Kentucky/UR06-0042/2007 (H1N1)
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A/Virginia/1/2006 (H1N1)
A/Arizona/1/2006 (H1N1)
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A/Washington/28/2007 (H1N1)
A/Florida/12/2007 (H1N1)
A/South Dakota/06/2007 (H1N1)
A/Managua/4086.02/2008 (H1N1)
A/New York/208/2001 (H1N1)
A/Pennsylvania/01/2008 (H1N1)
A/Texas/41/2007 (H1N1)
A/Colorado/33/2007 (H1N1)
A/New York/146/2000 (H1N1)
A/Memphis/15/1996 (H1N1)
(NIH) 2006 (H1N1)
A/Ohio/04/2006
A/Iowa/CEID23/2005 (H1N1)
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A/Swine/Quebec/5393/91 (SwQc91)
A/swine/Ontario/57561/03 (H1N1)
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A/muscovy duck/New York/21211-5/2005 (H1N
A/blue winged teal/TX/43/2002 (H1N1)
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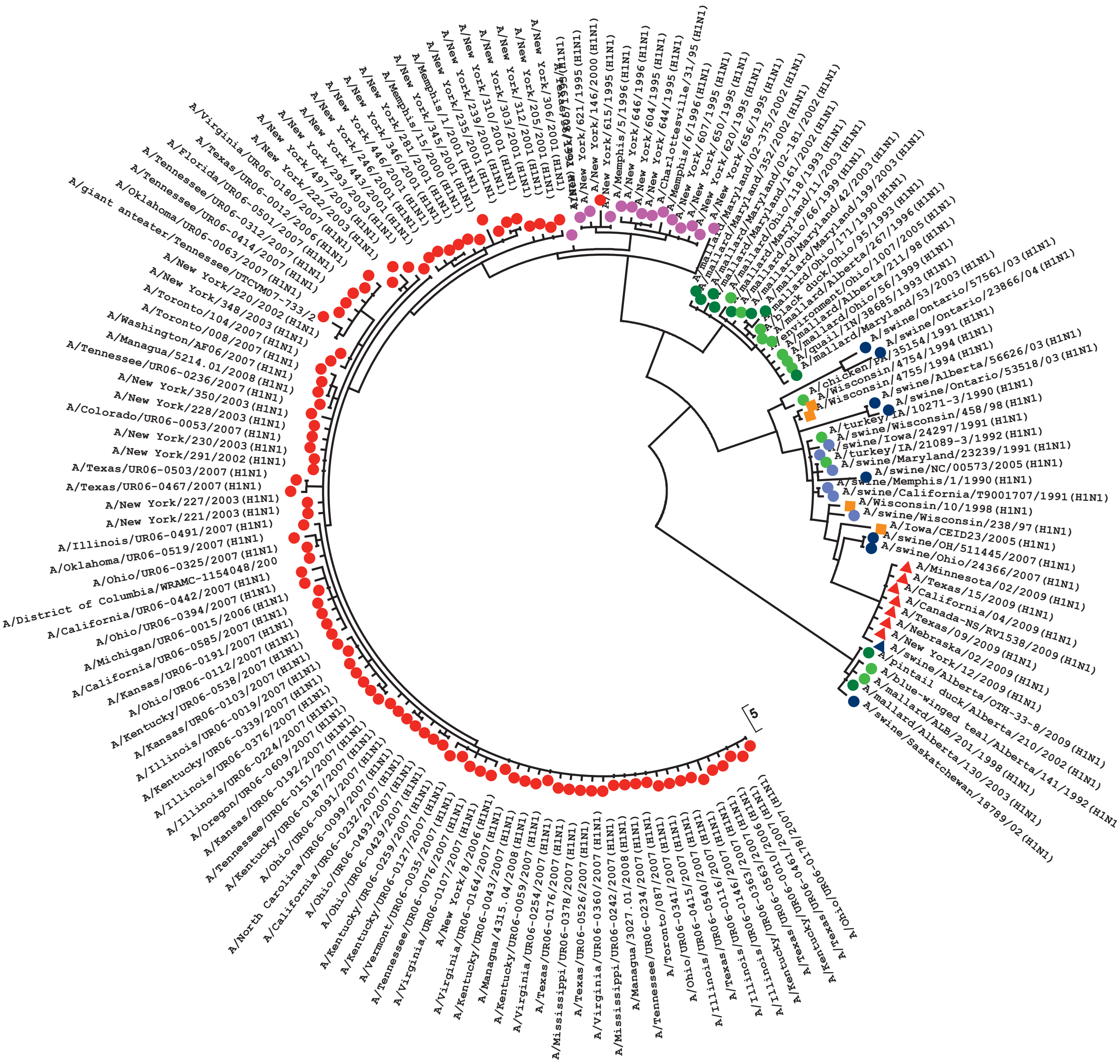
h **NS1**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A/turkey/IA/10271-3/1990 (H1N1)
A/swine/Wisconsin/458/98 (H1N1)
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A/swine/Memphis/1/1990 (H1N1)
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A/Iowa/CEID23/2005 (H1N1)
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A/swine/Ohio/24366/2007 (H1N1)
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A/Texas/15/2009 (H1N1)
A/California/04/2009 (H1N1)
A/Canada-NS/RV1538/2009 (H1N1)
A/Texas/09/2009 (H1N1)
A/Nebraska/02/2009 (H1N1)
A/New York/12/2009 (H1N1)
A/swine/Alberta/OTH-33-8/2009 (H1N1)
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A/mallard/ALB/201/1998 (H1N1)
A/mallard/Alberta/130/2003 (H1N1)
A/swine/Saskatchewan/18789/02 (H1N1)
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A/Washington/AF06/2007 (H1N1)
A/Managua/5214.01/2008 (H1N1)
A/Tennessee/UR06-0236/2007 (H1N1)
A/New York/350/2003 (H1N1)
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A/Colorado/UR06-0053/2007 (H1N1)
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A/New York/291/2002 (H1N1)
A/Texas/UR06-0503/2007 (H1N1)
A/Texas/UR06-0467/2007 (H1N1)
A/New York/227/2003 (H1N1)
A/New York/221/2003 (H1N1)
A/Illinois/UR06-0491/2007 (H1N1)
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A/Ohio/UR06-0112/2007 (H1N1)
5

Supplementary table 1. Closest protein homology of influenza 2009 A(H1N1) viruses

Gene	Best Hit	Identity	Lineage
PB2	A/Swine/Illinois/100085A/01 (H1N2)	98%	Swine
PB1	A/Wisconsin/10/98 (H1N1)	98%	Human
PA	A/Swine/Illinois/100084/01 (H1N2)	98%	Swine
HA	A/Swine/Indiana/P12439/00 (H1N2)	95%	Swine
NP	A/swine/Guangxi/13/2006 (H1N2)	98%	Swine
NA	A/swine/Spain/WVL6/1991 (H1N1)	94%	Swine
MP1	A/swine/Laer/IDT4126/05 (H3N2)	99%	Swine
NS1	A/SW/IN/14810-T/01 (H1N2)	94%	Swine

Supplementary table 2. Closest protein homology of influenza 2009 A(H1N1) viruses with swine influenza viruses that have infected humans

Protein	Cases	Identity	Lineage	Reference
PB2	A/Iowa/CEID23/2005(H1N1)	98%	Human	[1]
PB1	A/New York/727/1994(H3N2)	98%	Human	ABG48024
PA	A/Ontario/RV1273/2005(H3N2)	97%	Human	[2]
HA	A/Wisconsin/10/98 (H1N1)	93%	Human	AAO88265
NP	A/Iowa/CEID23/2005(H1N1)	97%	Human	[1]
	A/MD/12/1991(H1N1)	98%	Human	AAA51491
	A/Ohio/3559/1988(H1N1)	98%	Human	ABU80404
	A/Ohio/3523/1988(H1N1)	97%	Human	AAA73104
NA	Novel protein	Nf	Nf	Nf
MP1	A/Hong Kong/1774/99(H3N2)	99%	Human	[3]
NS1	/Wisconsin/10/1998(H1N1)	93%	Human	AAO88260
	A/Ontario/RV1273/2005(H3N2)	94%	Human	[2]

Nf = Not found

Supplementary table 3. Reported cases of pig-human interspecies transmission of influenza A (H1N1) occurred in Iowa, Maryland and Wisconsin, USA between 1991 and 2006. These influenza virus subtypes possess genetic distinctness compared to main cluster of human influenza A (H1N1) viruses

Influenza A virus subtype	Evidence linking pig-human infection	Reference
A/Iowa/CEID23/2005 (H1N1)	Yes	[1]
A/Wisconsin/10/1998	Insufficient data	[4]
A/Wisconsin/4754/1994	Yes	[5]
A/Maryland/12/1991	Yes	[5]
A/MD/12/1991	Yes	[5]
A/Wisconsin/4755/1994	Yes	[5]
A/Wisconsin/87/2005	Yes	[6]
A/Iowa/01/2006	Yes	[6]

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Norwegians approve of the health authorities' strategy to communicate worst case pandemic scenarios

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According to the Norwegian pandemic preparedness plans, health authorities shall assess their communication activities before and during an outbreak of infectious diseases. A survey was conducted on 29 April 2009 on acceptance of communications by the national public health authorities concerning the emerging threat from the new influenza A(H1N1) virus. The survey was similar to other surveys in 2005-6 about the avian flu. The results were not very different – the overall majority of the people interviewed were not worried and the health authorities were regarded as trustworthy.

Introduction

Norwegian media coverage (broadcast and press) of the new influenza A(H1N1) virus outbreak in Mexico and the Unites States rose markedly in the days following the World Health Organization's (WHO) alert on 24 April 2009 [1] and a substantial number of domestic news articles were registered. Spokespersons talking at daily news briefings on behalf of Norway's health authorities did not rule out the worst case scenarios laid down in the National Pandemic Contingency Plan. Thus, the possibility of a severe pandemic caught the headlines which warned that the number of deaths might equal that of the Spanish flu 90 years ago. A further focus of the media reports was on public preparedness measures and advice to the public.

In order to evaluate the plans for a future communication strategy and to assess the public relations work done from 24 April to 29 April, a survey was conducted on 29 April 2009 by one of the largest public research companies in Norway, Synovate Research. The research was done on behalf of the Norwegian health authorities and it took place in the hours just before WHO raised the phase of pandemic alert level from phase 4 to phase 5.

Methods and results

The survey was conducted following standard procedures by picking phone numbers randomly from the telephone directory. A total of 1,368 Norwegians were contacted and 506 (37%) interviewed, weighted according to age, sex and geographical location to make the selection representative. They were given the following possible answers to each of the six statements enumerated below:

- I completely agree or partially agree
- I neither agree nor disagree
- I partially disagree or totally disagree
- I don't know / cannot answer

The following passage presents the results for each statement.

"I am not worried about catching the 'swine flu' now." Eight out of 10 Norwegians stated that they are not worried.

"I feel confident that Norwegian health authorities are well prepared for a possible 'swine flu' outbreak with human-to-human transmission in Norway." Eight out of 10 Norwegians are confident that the authorities are well prepared.

"Norwegian health authorities have provided good and balanced information about the 'swine flu'." Seven out of 10 respondents consider the authorities have provided good and balanced information.

"Norwegian health authorities have exaggerated the danger related to the 'swine flu'." Five out of 10 participants do not think the authorities have exaggerated the dangers.

"Outbreaks, such as the 'swine flu', should be taken seriously because one never knows when a dangerous flu pandemic will break out." Nine out of 10 agree that these outbreaks should be taken seriously.

"There is too much media focus on the 'swine flu'." Six out of 10 Norwegians think there is too much media focus on the topic.

Conclusions

Similar surveys on the perception of the Norwegian citizens on the communication activities of the health authorities were conducted in 2005 and 2006 concerning the avian flu. The maximum press coverage on this public health event was in February-March 2005 with a focus on worst case pandemic scenarios. There were 20% more articles about bird flu registered in the domestic press during that period than during the influenza A(H1N1) outbreak so far. The answers were more or less in line with this year's survey.

Our surveys are examples of what health authorities can do to monitor the impact of their communication efforts on national public opinion. As all opinion polls, they are a snapshot valid for

a particular context, time and space. However, at the time of the surveys, Norwegians seemed to be open to listening to worst case scenarios and have confidence in the authorities.

The data presented from the survey allow for further comments. Surveys like these may be useful when planning risk communication strategies [2]. Further research on the topic should be inspiring for health authorities in our as well as other countries.

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Autochthonous cystic echinococcosis in patients who grew up in Germany

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Human cystic echinococcosis (CE) is a widespread zoonosis. Cases occurring in Germany are considered to result from imported infection and it is unclear if Echinococcus granulosus (sensu lato) is still transmitted in Germany. Therefore, exposure was investigated in 15 patients with cystic echinococcosis (7 female, 8 male; age-range 16-68, with a median of 48 years) who grew up in Germany. Fourteen patients had most likely acquired their infection in rural Germany, 11 from local dogs, one from an imported dog, two without obvious dog contacts. Taking into account multiple conceivable confounding factors might also account for some of infections: contacts with imported dogs or contact with dogs during travel in highly endemic regions, and ingestion of food contaminated by worm ova, whether in Germany or abroad. However, in at least two cases autochthonous transmission is beyond doubt, because these patients had never left Germany. The long pre-symptomatic development of cystic echinococcosis does not allow for a precise evaluation of the actual epidemiological situation. Compulsory notification of human cystic echinococcosis is an important instrument in the surveillance of the disease in humans. Regular inquiries at laboratories carrying out work in the field of veterinary medicine and at slaughterhouses, supervision of dogs at risk as well as genetic investigations on the strain or species of the causal agent of cystic echinococcosis are needed.

Introduction

Echinococcosis is a zoonosis occurring worldwide. Two forms of echinococcosis can affect humans: alveolar and cystic echinococcosis. The causal agent of alveolar echinococcosis is *Echinococcus multilocularis*. It is found in foxes, dogs, cats and wolves. The main host, the fox, contracts *E. multilocularis* mostly from eating rodents. *E. multilocularis* is known to sporadically transmit to humans in Germany [1, 2]. The domestic dog is the most frequent main host for *E. granulosus* and life cycles occur between dogs and different domestic animals including sheep or pigs. Worldwide human cystic echinococcosis following infection with several forms of the heterogeneous *E. granulosus* complex, accounts for most cases of human echinococcosis. The worldwide incidence of cystic echinococcosis is estimated to amount to 100,000 to 300,000 cases annually [3, 4]. Pastoral populations in East Africa, Kazakhstan, Kyrgizstan, northwest-China and Tibet are particularly at risk. In Europe, human infections occur predominantly in the south and east [3, 4].

In Germany cystic echinococcosis was known to be transmitted autochthonously until the sixties. Nowadays, however, cystic echinococcosis is perceived as an infection of migrants acquired in their countries of origin. Established transmission cycles are considered to have been interrupted in Germany by the improvement of hygiene in slaughterhouses, preventing the access of dogs to infected organs of slaughtered animals. Sporadically, cystic echinococcosis is registered in German individuals, but the high mobility of the population and the long-lasting pre-symptomatic phase precludes the possibility of reconstructing where the infection had been acquired.

At present, it is not clear, whether or not transmission of cystic echinococcosis to humans still occurs in Germany. Since 2001, cases of cystic echinococcosis are a mandatorily notifiable disease that needs to be reported to the Robert Koch-Institut (RKI, German national public health institute). Between 2001 and 2007 some 413 of notified cases were identified as new infections. Notifying doctors communicated the most probable source of infection in 296 of these 413 cases. Among these 296 cases more than one sixth (56 cases) of infections were deemed as having been acquired in Germany [5]. Therefore, we attempted to identify particular risks and the most probable source of infection by conducting a survey among patients with cystic echinococcosis who grew up in Germany.

Patients, materials and methods

Patients were recruited among individuals diagnosed with cystic echinococcosis, who grew up in Germany, and attended our regional referral centers in Germany, between 1999 and 2008. Patients were given detailed information on the study and asked for their consent to participate. Criteria for the definite diagnosis of cystic echinococcosis were imaging findings (ultrasound, computerized tomography and magnetic resonance imaging) showing a typical morphology for cystic echinococcosis. The findings were, classified according to the recommendations of the World Health Organization (WHO) - Informal Working Group on Echinococcosis [6]. Patients with transitional partially solidified cysts (WHO-CE

4) are sometimes difficult to diagnose on imaging findings alone: these cases were only included when other parameters (histology, detection of hooks or protoscolices in cystic fluid, antibodies to E. granulosus) supported or confirmed the imaging findings [7-9] (Table). Treatment and follow up were performed according to the stage of the disease [3,7,9].

Patients were asked to answer a detailed questionnaire concerning their entire life history and living conditions in all places where they had lived, with emphasis on urban or rural environment, dog contacts, whether they knew if slaughtering was controlled or not in the area they lived, and possibilities of an accidental transportation of parasite ova from dog faeces to raw food by cockroaches or flies. Patients were asked to present a detailed life-long travel history answering the same questions as in the questionnaire above. Where patients reported contacts to dogs, information on the origin and history of displacements of the dogs was also obtained.

Results

History of exposure

Twentytwo patients with cystic echinococcosis, who had grown up in Germany, were recruited for the survey. Seven of them were excluded because their data were incomplete. The 15 remaining German cystic echinococcosis patients, seven female, eight male, were able to give exhaustive information to answer the questionnaire. Their age at the date of diagnosis was 16 to 68 years (median 48 years). Detailed results on their history of travel and exposure to dogs and findings (laboratory and imaging) are shown in the Table. Since patients were uncertain about possible transmission risks, other than the two mentioned above, the cumulative duration of dog contacts in and outside Germany was defined as the best measurable risk factor.

Only two patients (n° 4 and n° 6) did not recall contacts with dogs. These two patients mainly had lived in Germany, although one patient (n° 4) had stayed for some months in a high risk area, Northern Africa, the other patient (n° 6) had travelled in areas with a high incidence of cystic echinococcosis only on holidays.

Among the patients who recalled having been in contact with dogs, one patient (n° 9) reported an extended stay in a rural area of central Italy, where he had kept dogs. For many years he also owned dogs in Germany.

Two patients (n° 13 and n° 14) had never left Germany even for short periods. For 10 patients the cumulative time of exposure to dogs was longest in Germany. One of them, patient n° 11, had imported his dog from Hungary to Germany, whereas the others had been exposed to local dogs only. Some of the latter patients may have also occasionally been exposed to cystic echinococcosis outside Germany, such as patient n° 8, a medical doctor, who had worked in Brazil for four years. However, he had lived in an area of very low endemicity and he did not recall any contact with local dogs there [4; 10]. Although patient n° 7 had lived for some time in highly endemic regions he did not remember any contact with dogs during these stays. Five of the patients reported only short holidays in endemic countries but did not remember any contact with local dogs (n° 2, n° 5, n° 10, n° 12, n° 15). Patient n° 3 had taken her pet dog with her on holidays to Italy (Riccione, Emilia- Romagna).

Discussion

Unexpectedly, in the majority of cases included in our study, infection by a local dog was the most likely explanation of cystic echinococcosis in patients who grew up in Germany. In two of the 15 cases there is no doubt about autochthonous infection, because they have never in their life-time travelled outside Germany. Our hypothesis of autochtonous infection in Germany may be confuted in some other cases where infection might also be interpreted as a travel associated disease [11].

The probability of autochthonous transmission depends on the prevalence of cystic echinococcosis in domestic animals, on the access of dogs to raw slaughter offal or to infected animal carcasses and the intensity and duration of contact between dogs and humans. Dog ownership, in particular the duration of dog ownership is the best established risk factor for human cystic echinococcosis [12]. Sometimes, humans may become infected without contact to dogs; indirect transmission occurs, when arthropods such as flies or cockroaches or birds transport ova of *E. granulosus* from dog excrements on raw food, e.g. salad [12-18]. In a rural environment, small children may also become infected when they accidentally ingest ova after crawling on the floor which has been contaminated by excrements of an infected dog. The type of water supply (i.e. tap water, wells) has also been suggested to be associated with the risk of human cystic echinococcosis. In a highly endemic rural area of Kazakhstan five out of 120 selected soil samples contained eggs of *E. granulosus* [19]. Obviously, no patient in our series could exclude these conceivable indirect ways of transmission. Indirect transmission most likely accounts for those two of our patients who did not recall any dog contact and may account for some other case, although an occasional dog contact which has been forgotten cannot be ruled out completely. However, the risk of indirect transmission by such sporadic events appears to be much lower than a long-lasting contact to a dog that is harbouring adult worms and thus constantly excreting worm ova over a time period of up to 22 months [10, 12-18].

Unfortunately, in Germany reliable data on the actual prevalence of cystic echinococcosis in domestic animals are not available. Infections of cattle were sporadically reported in Germany until the nineties [10; 20]. The prevalence of *E. granulosus* infections in dogs is assumed to be very low. The only vertical analysis available revealed 43 *E. multilocularis* cases but no E. granulosus confirmed by molecular analysis out of more than 21,000 specimens of dog excrements sent by Veterinary Medical Clinics to a German Veterinary Medicine laboratory in 2004 and 2005 [21]. This observation, however, cannot be taken as representative, because it can be assumed that rural free raging dogs are grossly underrepresented in this sample.

Controlled slaughtering and inspection of meat, as well as routine deworming of dogs have contributed to an almost complete disappearance of *E. granulosis* in Germany and many neighbouring countries. However, active foci are still present in countries close to Germany and frequently visited by Germans such as Poland and the Mediterranean countries [10; 20; 22].

A persistence of a reservoir of cystic echinococcosis in Germany cannot be excluded and new risks may arise, such as importation of infected dogs from endemic areas without deworming as well as the illegal slaughtering of domestic animals. Recently the re-introduction of cystic echinococcosis to slaughterhouses of a non-

endemic country has been observed in the Netherlands, where infected cattle had been imported from Romania [23].

As a possibility to more reliably identify the sources of human cystic echinococcosis in Germany, genetic investigations of the parasite could be helpful. In former times, cystic echinococcosis of domestic animals in Germany most frequently occurred in cattle. It can therefore be assumed that cystic echinococcosis in Germany was due to the genetically distinct cattle strain (G5 or *E. ortleppi*). An old persisting endemicity would be due to this agent. Genotypic analysis could indicate the origin of the infectious agent: sheep- and buffalo strains (G1/G3) are endemic in the Mediterranean region, and the pig strain (G7 or *E. canadensis*) is endemic in eastern Europe [9; D'Amelio, personal communication, 2007].

The actual risk of transmission is very difficult to determine because of the very slow development and persistence of cysts in patients for years or even decades. Considering the size and morphology of the cysts in our patients, infection must have taken place many years before diagnosis. The two older patients, who had never travelled outside Germany and who had inactive cysts, might have been infected in a period before cystic echinococcosis control had been completely achieved in Germany. This view may be supported by observations from other countries where cystic echinococcosis had been eradicated in animals but cysts were found in humans for many years after transmission had been interrupted [24,25]. To fully exclude sporadic infection acquired in endemic regions in the younger patients is impossible. Nowadays, it is difficult to find young Germans who have never travelled abroad and infections which are transmitted at present will most probably be discovered in humans only after years or even decades.

Furthermore, false negative serology results occur frequently, especially in young cysts (WHO-CL, WHO-CE1) and inactive cysts (WHO-CE5) [3, 6-10, 13, 17, 26, 27]. Obviously, laboratory notifications miss those cases where specific antibodies are not yet detectable. An inquiry among German pathologists showed a number of approximately 70 new cases of cystic echinococcosis detected each year, between 1995 and 2001 [28]. In our series, histopathology had been performed in less than half of the cases. Moreover, some cases of cystic echinococcosis in German patients may be misclassified as alveolar echinococcosis, because the notion that alveolar echinococcosis is endemic in Germany is common, whereas cystic echinococcosis is considered a disease of migrants. Reporting clinicians may also overestimate risks of acquiring cystic echinococcosis abroad because they believe that it cannot be transmitted in Germany. Thereby, cystic echinococcosis cases estimated of having been acquired in Germany are likely to be underreported. Furthermore, differential diagnosis between congenital cysts and cystic echinococcosisis is sometimes difficult [29]. Finally, many medical doctors in Germany are not yet sufficiently familiar with the notification procedures and these have not found their place in medical routine. Therefore, the real number of cystic echinococcosiscases is likely to exceed the number of notified cases. This notion is supported by a recent study on alveolar echniococcosis where a threefold higher incidence was found compared with the national surveillance figures [30].

The main limitations of our study are the impossibility to retrospectively assess transmission risks besides dog contacts and the high mobility of Germans with frequent stays and holidays in endemic areas. Nevertheless, despite of these difficulties, it must be acknowledged that autochthonous transmission of cystic echinococcosis in our patient series had occurred without doubt at least in two of 15 cases investigated and that the cumulative duration of dog contacts in the majority of the remaining patients was highest within Germany.

At present it is not possible to assess the actual risk of cystic echinococcosis transmission in Germany. The difficulties arise from: the very long delay between infection and diagnosis; the permanence of (apparently) inactive cysts which do not cause symptoms for years or even decades, insufficient data on the frequency of cysts found in slaughtered animals including the possible practice of uncontrolled slaughtering, and insufficient data on the incidence of E. granulosus infection in local dogs.

Conclusions

Cystic echinococcosis is a worldwide zoonosis, which occurs sporadically also in Germany. The results of our study strongly support the notion that a significant proportion of the sporadic infections are due to autochthonous transmission in Germany, although cystic echinococcosis may also be acquired from dogs imported from a region of high endemicity or during a stay in a highly endemic region. The retrospective design of our study does not permit an estimation of the actual risk of transmission of cystic echinococcosis in Germany. However, new threats have to be taken into account. The European Union is expanding and animal imports are likely to increase especially from highly endemic countries in Europe. Epidemiological figures on the actual transmission are difficult to obtain. Therefore, compulsory notification of human cystic echinococcosis is one instrument for surveillance that has to be maintained. Regular inquiries in veterinary medicine laboratories and slaughterhouses, investigation of dogs at risk of infection as well as genetic investigations on the strain or species of the causal agent of cystic echinococcosis are also justified.

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TABLE

Demographic data, history of exposure, clinical symptoms and findings prior to diagnosis in patients with cystic echinococcosis who grew up in Germany

Patient-n°. age at date of diagnosis (years)/ sex (m = male, f = female)	History of exposure						Diagnostic findings					
	Countries of exposure	Cumulative duration of stay	Pet contact	Cumulative duration of pet contacts (weeks)	Clinical symptoms	Conditions of discovery of Cystic echniocccois	Eosinophils/ µl (normal ≤400); (normal ≤4%)	IgE (IU/ml) (normal≤100)	Serology IHA* (<32^{-1})	Serology ELISA†	Other investigations	Imaging±
1 16 female	Germany (rural)	16 years	Frequent visit to grandparents who slaughtered domestic animals at home. Grand father also had CE	832	None	Accidentally by ultrasound	≤400/µl ≤4%	≤100	negative	n.a.§	IgE-CAP-RAST specific for CE¶ Histopathology positive	Liver cyst CE 1
	Italy	3 weeks	n.a.	n.a.								
2 19 female	Germany (rural))	19 years	Dogs in the neighbourhood	988	Abdominal discomfort	By ultrasound because of abdominal complaints	100/µl 1.9%	365	Positive	n.a.	Histopathology positive	active Liver cyst
	Italy (Sardinia)	2 weeks	None	None								
	Morocco	2 weeks	None	None								
3 27 female	Germany	22 years	Own dogs	988	None	Accidentally by ultrasound	282/µl 3.0%	18	Positive	Positive	# CBR neg **IFT neg Histopathology positive	Liver cyst CE 5
	Croatia(beach)	3 weeks	None	None								
	Italy (beach)	3 weeks	Brought her pet along from Germany	3								
4 38 male	Germany	38 years	None	None	Severe back pain	Accidentally by ultrasound performed during a dissection of a renal artery	≤400/µl ≤4%	33.5	Positive	n.a.	IgE-CAP-RAST specific for CE: 1 Western-Blot positive	Liver cyst CE4
	Newzealand	6 months	None	None								
	Northern Africa±±	5 months	None	None								
	Japan	10 weeks	None	None								
5 38 male	Germany (rural)	38 years	Yes	1872	Mild abdominal discomfort	Accidentally by a posttraumatic ultrasound after a ski accident	≤400/µl ≤4%	≤100	Positive	n.a..	IgE-CAP-RAST specific for CE: 0 Histopathology positive	Ruptured liver cyst with bacterial superinfection
	United States	10 weeks	None	None								
	Turkey	3 weeks	None	None								
	Libanon	1 week	None	None								
	Tunesia	1 week	None	None								
6 41 female	Germany (rural)	41 years	None	None	None	Accidentally by ultrasound	≤400/µl ≤4%	≤100	Positive	n.a.	IgE-CAP-RAST specific for CE: 1	Multiple liver cysts
	Spain (Canarian Islands)	3 weeks	None	None							Histopathology positive	
	Mediterranean	3 weeks	None	None								
	Yugoslavia (beach)	3 weeks	None	None								
	Italy(beach)	3 weeks	None	None								

7 47 male	Germany (rural)	18 years	Yes	936	None	Accidentally by ultrasound performed because of abdominal pain due to acute appendicitis	≤200 / µl ≤4.4%	211	Negative	Negative		Calcified kidney cysts, calcified spleen cysts
	Netherlands (rural)	4 years	None	None								
	Peru (rural)	8 months	None	None								
	France (rural)	2 months	None	None								
	Nigeria (rural)	1 month	None	None								
	Greece (rural)	1 month	None	None								
	Russia (urban)	1 Monat	None	None								
8 48 male	Germany (partially rural)	34 years	Yes	312	Cough	Chest X ray performed because of coughing revealed a pulmonary shadow	≤900 / µl ≤20,4%	3940	Positive	Positive	Western-Blot Positive Histopathology Positive	lLng cyst liver cyst CE 3
	Brazil (urban)	4 years	None	None								
	Portugal (urban)	2 months	None	None								
9 50 male	Germany (rural)	37 years	Yes	1508	Mild abdominal discomfort	Accidentally by ultrasound	≤100 / µl ≤0.9%	184	Negative	Negative		Liver cyst CE 4
	Italy (rural)	6 years	Yes	312								
	Spain (rural)	4 months	Yes	18								
10 60 female	Germany (rural)	60 years	Dog of her brother	780	None	Accidentally by ultrasound	≤100 µl ≤2.5%	6	Negative	Positive		Calcified spleen cyst
	Austria (rural)	Once yearly for 1-3 weeks each	None	None								
	Spain (Canary Islands)	2 weeks	None	None								
	Switzerland	2 weeks	None	None								
	Netherlands	2 weeks	None	None								
	France	2 weeks	None	None								
	Turkey	2 weeks	None	None								
	Greece	2 weeks	None	None								
11 63 male	Germany (mostly rural)	41 years	Yes	1334	Dyspnea	Accidentally by ultrasound performed for differential diagnosis of acute atrial fibrillation	≤200 / µl ≤2,1%	19	Negative	Positive		Liver cyst CE 2
	Hungary (hunting)	2 weeks	Yes, own dog imported from Hungary	624 (contact Continued in Germany)								
	Austria (hunting)	2 weeks		2								
	France (urban)	12 days	None	None								
	Portugal (beach)	7 days	None	None								
	Greece (beach)	5 days	None	None								
	Netherlands	7 days	Yes	1								
	Yugoslavia (beach)	5 days	None	None								

12 66 female	Germany (rural)	66 years	Yes	728	None	Accidentally by radiology showing a splenic calcification	≤100 / µl ≤1,1%	38	Negative	ND********		Calcified spleen cyst
	Spain (rural)	8 days	None	None								
	Italy (rural)	8 days	None	None								
13 67 male	Germany (rural)	67 years	Yes	3328	None	Epigastic pain,, palpable abdominal mass	≤200 / µl ≤3,4%	46	Positive	Negative		Liver cysts CE 1
14 67 female	Germany (rural)	67 years	Yes	2964	Abdominal discomfort	Increased liver enzymes during routine screening	≤55,7 / µl ≤1%	10,3	Positive	Positive	IgE-CAP-RAST specific for CE: 3 Histopathology Positive, Microscopy of cyst fluid (hooks, protoscolices)	Multiple iver cysts in different development stages
15 68 male	Germany (rural)	26 years	Yes	1144	None	Accidentally by a posttraumatic ultrasound screening after a ski accident	≤100 / µl ≤3,4%	130	Positive	Positive	Western Blot positive	Liver cyst CE 5
	India (holiday trip)	3 weeks	None	None								
	Spain (Canary Islands, beach)	3 weeks	None	None								
	Republic of South Africa (beach)	2 weeks	None	None								

±± Areas of high endemicity include: Mediterranean countries such as Portugal, Spain, Central and Italy,(southern part, Sardinia and Sicily,), France (southern part), former Yugoslavia including Croatia, Greece, Turkey, Lebanon; Northern Africa; Kazakhstan, Kyrgyzstan, Nepal, parts of China.
Areas of intermediate endemicity include: parts of Central Europe (Hungary, parts of France), South America, India, West Africa, southern Africa.
Areas with sporadic cases reported: Central Europe (Germany, Switzerland, Austria, Benelux, Scandinavia, United States, Mexico [4,10,12-18,20,22,26]
n.a. = not available,
IHA = immuno haemagglutination,
† ELISA = enzyme-linked immunosorbent assay
±Imaging: classification of liver cysts according to WHO [6];
CE = cystic echinococcosis,
§ n.a.*¶ CAP RAST = radioallergosorbent test [8];
#CBR = complement binding reaction,
** IFT = immunofluorescence test
††ND = not done