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Lack of evidence for presymptomatic transmission of pandemic influenza virus A(H1N1) 2009 in an outbreak among teenagers; Germany, 2009

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Running head: outbreak of A(H1N1) 2009 at a teenagers' party

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Abstract

Background: Observations on the role of pre-symptomatic transmission in the spread of influenza virus are scanty. In June 2009, an outbreak of pandemic A(H1N1) 2009 infection occurred at a teenager's party in Germany.

We performed a retrospective cohort study among party guests to identify risk factors for pandemic A(H1N1) 2009 infection.

Methods: Symptomatic pandemic A(H1N1) 2009 infection diagnosed by polymerase chain reaction between 1-14 June 2009 was defined as the outcome. Contact patterns among party guests were evaluated.

Results: In eight (36%) of 27 party guests the outcome was ascertained. A travel-returnee from a country with endemic pandemic A(H1N1) 2009 who fell ill towards the end of the party was identified as the source case. Party guests with pandemic A(H1N1) 2009 infection had talked significantly longer to the source case than non-infected persons (p-value: 0.001). Importantly, none (0/9) of those who had left the party prior to the source case's symptom onset became infected compared to 7 (41%) of 17 who stayed overnight (p= 0.06), and these persons all had transmission-prone contacts to the source case.

Conclusions: In this outbreak with one index case there was no evidence to support pre-symptomatic transmission of pandemic A(H1N1) 2009. Further evidence is required, ideally from larger studies with multiple index cases, to more accurately characterize the potential for presymptomatic transmission of influenza virus.

Key words: influenza A virus, H1N1 subtype; outbreak; cohort study; epidemiology; transmission; presymptomatic infectiousness

Introduction

After its first identification in Mexico in April 2009 the pandemic influenza virus A (H1N1) 2009 rapidly spread over all continents. On 11 June 2009, WHO raised the pandemic alert from level 5 to 6, marking the official beginning of the 2009 influenza pandemic.

Symptom-based interventions, such as isolation of cases, contact tracing and quarantine, were important public health measures to contain infection and delay spread at the early stages of the 2009 pandemic [1-3]. Their success may be limited if a substantial proportion of transmissions occurs through apparently healthy individuals (presymptomatic or asymptomatic) [4]. To our knowledge, there are no experimental or controlled studies, and only one observational study on presymptomatic transmission [5]. Therefore, viral presence in the upper respiratory tract is used as a proxy to infer infectiousness, also of symptom-free individuals [6-9]. However, even in these studies, data on viral shedding in the presymptomatic phase are scanty, and the relationship between nasopharyngeal virus detection and transmission is uncertain [7].

We report on the investigation of a pandemic A(H1N1) 2009 outbreak in a confined setting of a teenager's party in Germany in June 2009, where most of the exposure time was during the source case's presymptomatic period.

Methods

Outbreak setting

A group of 28 teenagers celebrated a party for two female teenage friends on 31 May 2009. The two friends had returned from Argentina - a country with community transmission of pandemic A(H1N1) 2009 at that time - two days earlier by air travel. By that time, less than 100 human cases, mostly travel-related, with confirmed pandemic A(H1N1) 2009 infection had been notified in Germany.

The party was held in a private house and lasted from 6 p.m. until the following morning. Eighteen party guests, including the two returnees, stayed overnight, the remaining nine left the

party between 11.30 p.m. and 1 June 2 a.m. One of the returnees (R1), female / 16 years of age, became symptomatic with influenza-like illness (ILI), defined as fever and cough or sore throat, on 1 June after 2 a.m. and stayed until 11.30 a.m. She was the first party guest with pandemic A(H1N1) 2009 infection confirmed by real-time reverse transcription-polymerase chain reaction (rRT-PCR) on 4 June. The other returnee (R2) had experienced symptoms of mild respiratory disease (coryza) since 26 May and was still mildly symptomatic during the party. She developed ILI on 2 June. After the diagnosis of pandemic A(H1N1) 2009 infection of R1 was reported to the local health authority on 4 June, the health officers contacted all party guests and asked about respiratory symptoms, fever or myalgia during the time since the party. Symptomatic individuals were tested between 4-7 June by nasal and throat swabs for pandemic A(H1N1) 2009 using rRT-PCR [10] performed at the Bavarian Health and Food Safety Authority, Oberschleissheim.

Cohort study

We conducted a retrospective cohort study among all party guests to identify the source case and characterize transmission risks. We defined a case as pandemic A(H1N1) 2009 infection confirmed by rRT-PCR who developed ILI between 1 and 5 June.

Between 13 and 22 June we administered a questionnaire to the party guests. The questionnaire covered demographical characteristics, symptoms at or after the party (e.g., fever, cough, sore throat, myalgia), potential exposures to pandemic A(H1N1) 2009 cases outside the party cohort, party attendance (time of arrival and departure) and information on duration and frequency of contacts during the party, which was assessed by the following variables: duration of talking (0 minutes, 1-14 minutes, 15-60 minutes, >1-4 h, >4 h) at ≤ 1 meter distance to each of the other party guests; frequency of hugging or kissing each of the other party guests (0x, 1-2x, 3-5x, >5x), and other contact types, e.g., staying overnight, sharing drinks, dancing with somebody. We obtained written informed consent of the participants' parents. Data were entered into an Epidata database (version 3.1) and analyzed using Stata® (v10.1 StataCorp, USA). We considered two-sided p-values <0.05 statistically significant.

Source identification: We hypothesized that one or both of the returnees were the source case(s) for the other party guests since they returned to Germany from a country, which had already reported community transmission of pandemic A(H1N1) 2009 at the time. We compared talking to and hugging or kissing R1 and R2 between infected and non-infected party guests using the Wilcoxon rank-sum test. We assumed that pandemic A(H1N1) 2009 infected persons had talked longer to the source case(s) and hugged or kissed her or them more often than uninfected persons. We also compared clinical manifestation, dates of symptom onset and dates of sampling between R1 and R2.

Risk factor analysis for pandemic A(H1N1) 2009 infection: Due to the small sample size and the presence of "zero cells", we employed bivariable exact logistic regression to compute odds ratios, 95% confidence intervals (CI) and two-sided p-values for all contact variables. To this end, we dichotomized the categorical contact variables (i.e. talking to the source case, hugging or kissing the source case) using their respective median as cut-off.

Results

Overall, 27 (96%) out of 28 guests participated in the study. Their average age was 16 years (range 15-19 years), 15 (55%) were girls.

Of 27 individuals, 25 could be contacted initially by the local health authority. Of these, ten (8 with ILI, 2 with symptoms not fitting the ILI case definition) were tested by rRT-PCR. All 8 individuals with ILI were positive. According to the questionnaires, four additional persons, of whom one had ILI, reported respiratory symptoms with onset during the outbreak period. In total, 9 ILI cases were ascertained, of whom 8 were tested and positive. Of 5 individuals with symptoms not fitting the ILI definition, two were tested and had a negative result.

Of 15 female individuals, 7 (47%) became cases compared to only one (8%) of 12 males (p=0.06).

<u>Source identification</u>

Cases (excluding the returnees) reported a significantly longer duration of talking to R1 and a higher number of hugs and kisses exchanged with her than non-cases (table 1). In addition, symptoms and timing of disease onset and sampling of R1 are fully compatible with influenza. By contrast, we found no significant differences with respect to the contact variables for R2 between cases and non-cases. She likely had two respiratory illnesses, of which the first, starting already on the 26 May, was not influenza-like. Furthermore, the positive sample was taken 9 days after onset of the first respiratory illness, which does not support an influenza infection at that time. In contrast, the second respiratory illness, occurring one day after disease onset of R1, was an ILI and it is highly likely to find influenza virus in an influenza-infected person 2 days after disease onset. Taken together, we concluded that R1 was the source case who infected also R2.

Incubation period

The date of contact with the symptomatic index case was 1 June. Dates of symptom onset for the other cases ranged from 2 June through 5 June, corresponding to an incubation period of 1-4 days with a mean of 1.5 days.

Risk factor analysis for pandemic A(H1N1) 2009 infection

Having identified R1 as the source case, we included R2 in the following analyses (n=26). All variables relating to R1 had increased odds for infection (table 2). Strength of association for becoming a case was highest for talking to R1 ≥ 15 minutes (OR 16.9, 95% CI 2.12 - +Inf), and kissing or hugging her more than twice (OR 11.6, 95% CI: 1.24 - 179.08). All party guests reported talking to the source case, but none of those who had talked to R1 <15 minutes became a case. None (0/9, 95% CI: 0-33%) of the party guests became a case who did not stay overnight and thus left before R1 developed symptoms, compared to 7 (41%) of 17 who stayed overnight (p= 0.06). Two out of the 9 who did not stay overnight developed an acute respiratory illness that did not meet the ILI definition, one of them was tested negative for pandemic A(H1N1) 2009 by rRT-PCR.

Discussion

In this investigation of a pandemic influenza outbreak among teenage party guests, we were able to identify a travel returnee from Argentina as the likely source for all other cases. The source case's symptom onset occurred after 2 a.m. which allowed us to categorize the exposure time during the party into a presymptomatic and a symptomatic period. Presumably, not all influenza cases are equally infectious [11]. However, this particular case apparently was highly capable of transmitting the virus (secondary attack rate 26.9%). Most notably, transmission was not observed among the nine party guests who were exposed only during the presymptomatic period. These persons all had transmission-prone contacts, which included talking to the source case for at least 1-14 minutes at a distance of less than 1 meter, as well as hugging, kissing, and likely, but unmeasured, passing her or dancing next to her several times.

Considering R1 as the only source case is plausible. She had just returned from a pandemic A(H1N1) 2009 endemic country and only contact variables relating to her were significantly associated with becoming a case - not for any other guest, including the other travel returnee. Furthermore, her symptom onset, the earliest of all pandemic A(H1N1) 2009 infected persons, was during the party, and the time interval between her symptom onset and that of secondary cases is in line with the incubation period derived from other outbreak investigations [12;13]. All secondary cases had contact to R1 during her symptomatic phase. Compared to non-cases, they had talked longer to the source case during the entire party and had had, anecdotally, also more intense contact to the source case (close friends). This likely applies also for the presymptomatic period and thus it remains unclear, at which point the cases became infected. More in-depth analysis (e.g. restricting analysis to those who had exposure during the symptomatic phase), would have necessitated collection of exposure information separately for the two periods (presymptomatic / symptomatic), which was not done. This is one limitation of our study. Furthermore, the accuracy of the exposure recall 2-3 weeks after the event may be different depending on the infection status. However the party was a rather unique and

memorable event for the participants in the light of heightened media attention of this first community outbreak of pandemic A(H1N1) 2009 in Germany, helping to minimize such differential recall. Lastly, we cannot rule out that further infections have occurred because we did not test all symptomatic persons. However, to estimate the influence of a possible incomplete outcome ascertainment, we repeated calculations using less specific outcomes, based on clinical definitions (influenza like illness and acute respiratory infection), but did not see a change for associated risks (data not shown). Nonetheless, caution should be exercised before generalising these results as the study was small in size, and confined to a particular setting and strain.

Data on pandemic A(H1N1) 2009 transmission are limited [14-17] as are, in general, data on transmission from pre-symptomatic exposure and on presymptomatic shedding in naturally acquired influenza infections. We are aware of only one study where presymptomatic transmission was investigated and seemed to have occurred [5]. Data on shedding of seasonal influenza virus during the presymptomatic period is sparse [6;7;14;18;19]. Pooled data from experimental voluntary influenza infections indicate that viral shedding precedes illness by about one day [20], and experience from naturally acquired infections suggests that only 1-8% of infectiousness occurs prior to illness onset [9]. In conclusion, our results from an outbreak with one index case do not support that pre-symptomatic infectiousness plays a role in pandemic influenza transmission. Further evidence is required, ideally from larger studies with multiple index cases, to more accurately characterize the potential for presymptomatic transmission of influenza virus.

Addendum

Individual contributions of authors

J Hermes 1,2,3,4,5; H Bernard 1,2,3,4,5; U Buchholz 4,5; M Spackova 1,5; J Löw 1,5; G Loytved 1,5; T Suess 1,4,5; W Hautmann 4,5; D Werber 1,2,3,4,5.

1 contribution to concept and design,

2 analysis and/or interpretation of data;

3 critical writing

4 revising the intellectual content;

5 final approval of the version to be published

Potential conflicts of interest

The authors do **all** not have a commercial or other association that might pose a conflict of interest.

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Table 1: Comparison of contacts to either of two travel returnees from Argentina (R1 and R2) during outbreak of pandemic influenza virus 2009 in a teenage party cohort; June 2009, Germany.

Contact exposure	cases	total	%	p-value
Talking to R1				0.004
0-1 min	0	0	0	
1-14 min	0	13	0	
15-60 min	2	6	33	
1-4 h	3	3	100	
>4 h	1	3	33	
Frequency of kissing R1				0.03
0x	0	2	0	
1-2x	2	16	13	
3-5x	2	3	67	
>5x	2	4	50	
Duration of talking to R2				0.35
0-1 min	1	1	100	
1-14 min	1	11	9	
15-60 min	0	5	0	
1-4 h	3	5	60	
>4 h	1	3	33	
Frequency of kissing R2				0.62
0x	0	1	0	
1-2x	3	13	23	
3-5x	2	8	25	
>3x	1	3	33	

Footnote: the denominator includes all party guests excluding R1 and R2;
P-value is given for the Wilcoxon rank sum-test.

Table 2: Risk factors[a] for infection with pandemic influenza virus in a teenage party cohort (n=26), June 2009, Germany:

Exposure variables	ill	total	(%, 95% CI)	OR	95% CI	p-value
gender				7.6	0.7 - 413.2	0.12
Female	6	14	(43, 18-71)			
Male	1	12	(8, 0-38)			
staying overnight				7.5	0.9 - +Inf	0.06
Yes	7	17	(41, 18-67)			
No	0	9	(0, 0-33)			
talking to the source case >=15 min[b]				16.9	2.1 - +Inf	0.005
Yes	7	13	(54, 25- 81)			
No	0	13	(0, 0- 25)			
kissing the source case > 2x[b]				11.6	1.2 - 179.1	0.014
Yes	5	8	(63, 24 – 91)			
No	2	18	(11, 1 – 35)			

[a] Using exact logistic regression. Displayed are variables with a p-value <0.1

Exposures with p-value >0,1: Close dancing, sharing drinks, contacts to any other party guest, contacts to an ill person outside the party, being at an international airport during the week prior to party, talking to R2, hugging and kissing R2

[b] Variables were dichotomized at their median values.