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Shedding and Transmission of Novel Influenza Virus A/H1N1 Infection in Households—Germany, 2009

Thorsten Suess*, Udo Buchholz, Susann Dupke, Roland Grunow, Matthias an der Heiden, Alla Heider, Barbara Biere, Brunhilde Schweiger, Walter Haas, and Gérard Krause on Behalf of the Robert Koch Institute Shedding Investigation Group

* Correspondence to Dr. Thorsten Suess, Department of Infectious Disease Epidemiology, Robert Koch Institute, FG 36, DGZ – Ring 1, 13386 Berlin, Germany (e-mail: SuessT@rki.de).

Essential epidemiologic and virologic parameters must be measured to provide evidence for policy/public health recommendations and mathematical modeling concerning novel influenza A/H1N1 virus (NIV) infections. Therefore, from April through August of 2009, the authors collected nasopharyngeal specimens and information on antiviral medication and symptoms from households with NIV infection on a daily basis in Germany. Specimens were analyzed quantitatively by using reverse transcriptase-polymerase chain reaction. In 36 households with 83 household contacts, 15 household contacts became laboratory-confirmed secondary cases of NIV. Among 47 contacts without antiviral prophylaxis, 12 became cases (secondary attack rate of 26%), and 1 (8%) of these was asymptomatic. The mean and median serial interval were 2.6 and 3 days, respectively (range: 1–3 days). On average, the authors detected viral RNA copies for 6.6 illness days (treated in time ¼ 5.7 days, not treated in time ¼ 7.1 days; P ¼ 0.06), but they estimated that most patients cease to excrete viable virus by the fifth illness day. Shedding profiles were consistent with the number and severity of symptoms. Compared with other nasopharyngeal specimen types, nasal wash was the most sensitive. These results support the notion that epidemiologic and virologic characteristics of NIV are in many aspects similar to those of seasonal influenza.

Abbreviations: CI, confidence interval; NIV, novel influenza A/H1N1 virus; RT-PCR, reverse transcriptase-polymerase chain reaction; SAR, secondary household attack rate.

In April 2009, novel influenza A/H1N1 virus (NIV) infections were first observed in Mexican and US American patients (1). Only weeks later, the virus had spread worldwide and, on June 11th, the World Health Organization announced phase 6 of pandemic alertness and thus the start of the 2009 influenza pandemic (2).

The basic clinical and epidemiologic characteristics of newly emerging pandemic viruses need to be assessed quickly, as these have implications on preventive strategies, clinical diagnosis, and epidemiologic modeling (3, 4). Regarding seasonal influenza, much of the knowledge on clinical manifestation and shedding was gained through experimental studies. Recently, a review of these studies has been published (5). Household studies represent another efficient study type that was used to examine basic influenza parameters in seasonal influenza, such as the serial interval (6), the duration of infectiousness (7), susceptibility and infectiousness of children versus adults (8), and the therapeutic and prophylactic effectiveness of neuraminidase inhibitors (9–13).

For NIV, some preliminary information, based on early—albeit limited—data, was already available, including generation time (i.e., duration of time between becoming infected and transmitting the virus to another person: 1.9 days (14) and 2.5–3 days (15)), serial interval (2.5 days (16), 2.9 days (17), 3 days (18), 4–5 days (19)), secondary household attack rates in contacts without antiviral prophylaxis (7.6% (18) and 26% (20)), and an apparently increased susceptibility of infection for children compared with adults (16–18).

We conducted a prospective, observational study of cases with NIV infection and their household contacts in Germany during the summer of 2009, in order to contribute information to the clinical and epidemiologic characteristics mentioned above.

The present study had been prepared and piloted in the interpandemic era as part of pandemic planning with the goal to rapidly collect important information on the properties of the pandemic virus upon the occurrence of the first cases in Germany.

Materials and Methods

When the first laboratory-confirmed pandemic cases were reported to the Robert Koch Institute, we immediately attempted to contact all case households via their respective state and local health departments to obtain verbal consent to conduct the study in the individual household.With a positive answer, the Robert Koch Institute sent out epidemiologic teams to the households of the confirmed cases. Written informed consent was obtained from all patients and their household members before enrollment. Patients and household members were visited either at home or, if hospitalized, in hospital. Enrollment lasted from April to August of 2009.

Case definition

The household index case was defined as the first person in the household with laboratory-confirmed NIV infection or as the first person with respiratory symptoms (without laboratory confirmation) when the disease of another person in the household was a laboratory-confirmed NIVinfection. Household secondary cases (with disease onset at least 12 hours after the index case) had to be laboratory confirmed.

Influenza-like illness was defined as fever plus ‘‘cough or sore throat.’’ All participants aged less than 14 years were defined as children, and all older participants, as adults. Laboratory confirmation was defined as a positive result of any specimen tested for NIV by reverse transcriptasepolymerase chain reaction (RT-PCR). We used the term ‘‘viral shedding’’ when RT-PCR detected viral RNA in a nasopharyngeal specimen. A household was defined as a domestic unit consisting of the members of a family who live together including nonrelatives and intimate partners (modified according to an online dictionary (21)). Participants living in one household with the respective index patient were termed ‘‘household members’’ or ‘‘household contacts.’’

Data and specimen collection

We assessed symptoms and antiviral medication from each household member on a daily basis starting from the day of symptom onset of the index case. Early in the study, we obtained the following specimens once a day from all household members in the following order: 1) nasal swab, 2) throat swab, 3) sputum, 4) throat wash, and 5) nasal wash. As it could only rarely be obtained from the participants, sputum was collected only occasionally. Later in the study, when the first results regarding the sensitivity of specimen types suggested a higher sensitivity for nasal wash, we took only nasal wash as a regular specimen. For the collection of nasal and throat wash, we used 5 mL of isotonic saline, which were instilled into the nostril or the mouth, respectively, and afterwards collected in a sterile cup (22). Nasal and throat swabs were collected by using virus transport swabs (Mastaswab; MAST Diagnostica, Reinfeld, Germany). Sputum was obtained after a deep cough and collected in a sterile cup. Samples were stored refrigerated (at a temperature of approximately 5°C) and analyzed as soon as possible.

Secondary attack rates

Secondary household attack rates (SARs) were calculated as the proportion of household contacts without antiviral postexposure prophylaxis becoming a case within 8 days after symptom onset of the index case. Households were excluded from calculation of the secondary household attack rate if the index case was isolated in the hospital during the period of time relevant for virus transmission. We recorded the date of the beginning of treatment (if any) and regarded treatment with neuraminidase inhibitors as ‘‘timely’’ if it began within the first 3 days of illness, that is, the day of symptom onset plus 2 days. As antiviral prophylaxis was not initiated according to a predefined protocol (as would be the case in a randomized controlled trial), the effectiveness of antiviral prophylaxis could not be calculated.

Serial interval

We defined the serial interval as the number of days between symptom onset of the household index case and symptom onset of the first secondary household case. Other secondary household cases

were only included in the calculation of the serial interval if their symptomonset occurred on the same day as the first secondary household case.

Sensitivity of specimen types

For the calculation of sensitivity of specimen types, a sampling day was called positive on the basis of 2 conditions: First, all 4 regularly obtained specimens (nasal swab, throat swab, throat wash, nasal wash) were collected on that day, and second, at least 1 of those 4 specimens tested positive on that day. This led to 28 positive sampling days, which served as the denominator. The numerator changed with each specimen type and was defined as the number of positive results in each specimen type. The sensitivity of a specimen type was expressed in percent.

Viral shedding, viral shedding profile, and symptom profile

The minimal duration of viral shedding in symptomatic patients was defined as the time between symptom onset and the last day a RT-PCR–positive specimen was taken. For the calculation of the duration of shedding, we excluded asymptomatic study participants and those whose specimens were negative in our laboratory.

In order to display summary curves of clinical symptoms over the course of illness, we calculated a daily symptom severity score on a 4-level scale from 0 (not present) to 3 (severe) for each of the following symptoms: fever/chills, cough, sore throat, and headache/myalgia. Thus, the daily score ranged from 0 points (no symptoms) to 12 points (all symptoms with maximum severity). If, at the end of the follow-up period, symptom data were missing but the last recorded score was 0, we assumed that the respective patient continued to be asymptomatic.

For the assessment of the viral shedding profile, we included participants who were laboratory confirmed and had a definite day of symptom onset. If one or more positive test results were followed by a final negative test result, we assumed that the following days also were laboratory negative. To compare viral load (expressed as the log10 of RNA copies/mL) with the symptom score, we performed 2 calculations: 1) pooled analysis (for each illness day, we plotted the median of the log10 of RNA copies/mL against the median of symptom scores); 2) individual analysis (using a mixed-effect model described in ‘‘statistical analysis’’ below, we estimated the proportion of the variance of the log10 of RNA copies/mL that is explained by the daily variation of symptom score).

Laboratory methods

Specimens were analyzed for NIV by RT-PCR at the Centre for Biological Safety, Division of Highly Pathogenic Microorganisms (ZBS2), at the Robert Koch Institute. RNA was extracted by using either the RTP DNA/RNAVirus Mini Kit (Invitek Gesellschaft für Biotechnik & Biodesign mbH, Berlin, Germany) or the MagAttract Viral RNA M48 Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s suggestions. Details about the polymerase chain reaction protocol, as well as primer and probe sequences, are available upon request. Quantitative results were expressed as RNA copies/mL. A sample of specimens with a high number of RNA copies/mL was also tested for quantification of infectious viruses by using a plaque assay in a MDCKSIAT1 cell culture monolayer (23, 24). The results are presented as plaque-forming units/mL of specimen.

Statistical analysis

We used Student’s t test, chi-squared test, and Fisher’s exact test as appropriate. All statistical tests were 2 sided, and probability less than 0.05 was considered statistically significant. For the association of the daily viral load (RNA copies/mL) with the symptom score, we constructed a linearmixed-effects model with random slope and intercept on the individual level (25). Statistical tests were done with STATA, version 10, software (StataCorp LP, College Station, Texas).

Ethics approval

Ethics committee approval for the study was granted by the Ethics Committee of the Charité Universitätsmedizin Berlin (EA1/043/07).

Results

We included 36 households with 119 individuals (36 index cases and 83 household contacts). Of the 36 index cases, 30 were laboratory confirmed. Of the 83 household contacts 15 became secondary cases.

Characteristics of cases and noncases

Index cases displayed symptoms (fever, cough, sore throat, and headache/myalgia) more frequently than did household secondary cases with or without antiviral prophylaxis (Table 1). For headache/myalgia, this difference between index cases and secondary cases without antiviral prophylaxis was statistically significant (P ¼ 0.02). Oseltamivir was the only neuraminidase inhibitor used in this setting. Three household secondary cases were asymptomatic: 2 (67%) among the 3 cases with prophylaxis and 1 (8%) among the 12 cases without prophylaxis.

Household index cases were treated more frequently with oseltamivir compared with secondary cases who had not received antiviral prophylaxis before their onset of symptoms (56% vs. 25%) (Table 1). Two (6%) of 36 household index cases were hospitalized because of severity of illness but recovered; none of the 12 symptomatic secondary cases was hospitalized. Cases treated with oseltamivir within the first 3 days of illness were more severely ill at symptom onset than those who started oseltamivir later or not at all (influenza-like illness in timely treated patients: 93% (13 of 14); influenzalike illness in patients without therapy or without timely therapy: 57% (21 of 37)(P ¼ 0.02). There was no apparent difference in length of symptomatic disease between the 2 groups (treated timely vs. not timely/not at all) as the average symptomscore of both groups dropped to 1 or belowby day 9.

Secondary attack rates

The SAR among household contacts who had not received antiviral prophylaxis was 26% (12 of 47). The SAR among index cases aged less than 14 years was similar to that among adult index cases (4 (17%) of 24 vs. 10 (20%) of 49; relative risk ¼ 0.82; P ¼ 0.70). Susceptibility to NIV infection in secondary cases did not differ by age, as SAR in secondary cases among children (4 (36%) of 11) was not statistically different from that among adults (10 (16%) of 62; relative risk ¼ 2.25; P ¼ 0.12). Adjustment for other variables did not alter the result substantially.

Serial interval

Eight secondary cases provided information for calculation of the serial interval that ranged as follows: 1 day (1 patient), 2 days (1 patient), 3 days (6 patients). Thus, the median serial interval was 3 days (range: 1–3), and the mean was 2.6 days (standard deviation: 0.7).

Sensitivity of specimen types

Specimen type sensitivity was determined from 28 positive sampling days with at least 1 of the 4 specimen types yielding a positive RT-PCR result. Thirteen (46%) of the 28 positive sampling days were situated between the second and fifth days of illness, compared with 15 (54%) positive sampling days between illness days 6 and 9. Nasal wash yielded the highest sensitivity for detection of NIV (25 positive nasal wash samples (89%) of 28 positive sampling days, 95% confidence interval (CI): 72, 98). All other tested materials (nasal swab: 39%, 95% CI: 22, 59; pharyngeal wash: 29%, 95% CI: 13, 49; and pharyngeal swab: 21%, 95% CI: 8, 41) were less sensitive.

Viral shedding, viral shedding profile, and symptom profile

There was only 1 symptomatic case who was sampled for NIV before symptom onset. This case was sampled on the 2 days before symptom onset and tested positive on both days. All other secondary cases were sampled the first time on or after the day of symptom onset.

The mean minimal duration of viral shedding was 6.6 days in all symptomatic cases (standard deviation: 2.6 days) (Figure 1), 6.3 days in children, and 6.7 days in adults (children vs. adults, P ¼ 0.66). The mean duration of viral shedding in patients with timely oseltamivir treatment was 5.7 days compared with 7.1 days for those without (P ¼ 0.06).

Based on pooled data, the shedding profile corresponded well with the clinical course (Figure 2; Table 2). At least half of all symptomatic patients returned to a symptom score of 1 by day 6 and became asymptomatic by day 9. The lowest viral load values are 2 log10 RNA copies/mL, that is, 100 RNA copies/mL.

Analysis of viral load and symptom score by person-day using the mixed-effect model showed that 51% of the variance of the viral load can be ''explained'' by allowing the height of the viral load at disease onset of each case as well as the slope to vary randomly. Comparison of the log of the RNA copies/mL with the log of the plaque-forming units/mL showed that viral load needed to reach levels of at least 104 RNA copies/mL before viral culture became positive. It can be roughly estimated that the majority of symptomatic patients will cease to have detectable virus by the fifth day of illness (Figure 2).

Discussion

This study presents data concerning aspects of clinical manifestation, viral shedding, and transmission of NIV that are necessary for public health policy/recommendations and modeling.

Symptoms

All symptoms occurred less frequently in secondary household cases (without antiviral postexposure prophylaxis) compared with index cases. Although the number of secondary cases in our sample was small, it is likely to give a more accurate picture on the frequency of clinical symptoms and asymptomatic infections compared with information retrieved, for example, from surveillance data or—as in our study—index cases. The reason for this is that our prospective identification of cases among household contacts is likely to be less biased by surveillance case definitions and other forms of ascertainment biases.

Fever >38°C (67%) and cough (75%) were still the most frequent symptoms in secondary cases without antiviral prophylaxis, while sore throat (25%) and headache/myalgia (33%) were observed in only about a quarter of them. The World Health Organization case definition for influenza-like illness (fever >38°C, as well as cough or sore throat) would have captured only 58% (7 of 12) of secondary cases among contacts without prophylaxis in this study.

We found that, among contacts without prophylaxis, 1 (8%) of 12 secondary cases shed virus but did not display any symptoms. In a review of volunteer challenge studies of seasonal influenza, about one third of infections have been shown to be asymptomatic (5).We may have missed asymptomatic shedding if it occurred over a short period of time, because in several households we began sampling only several days after the respective index cases' symptom onset.

Secondary attack rates

The household SAR was 26% among contacts who did not receive antiviral prophylaxis. This value is identical to that from a study in Kenya (20) and consistent with data published by the World Health Organization (15), but it is substantially higher than those reported in studies from Japan (18) (7.6%in

household contacts who did not receive antiviral prophylaxis), the United Kingdom (16) (8.1%), or the United States (17) (13%). Treatment of index cases, climate differences, behavioral differences such as the use of face masks, and methods of identifying or defining secondary cases may have contributed to the differences between these studies and our data. It is known from seasonal influenza that SAR can differ by country, season, or study design; published values varied from 6% (26), 19%(13), and 23%(12) to 38%(27). Although not statistically significant, the increased SAR among children in our study is consistent with the results of several other NIV studies (16–18).

Serial interval

Within our small sample of secondary household cases, we report a median serial interval of 3.0 days. The serial interval indicates how quickly an epidemic can evolve. Thus, it can be an important parameter for mathematical modeling. Published estimates on the serial interval of NIV (mean: 2.5 days (16); mean: 2.6 days (17); median: 3 days (18)), which were calculated by using the same methods as applied in this study, resemble our data and are similar to estimates from seasonal influenza (6, 28). An exception is the study by Tuite et al. (19) that, by using a heuristic algorithm, estimated the serial interval for NIV to be 4–5 days.

Sensitivity of specimen types

This study demonstrates that the nasal epithelium appears to be the preferred anatomical location for sample collection and that nasal wash appears to be the most sensitive specimen type. Similar results have been obtained for seasonal influenza (29, 30). The substantial difference between nasal wash and the other specimen types in our study may have been enhanced by the fact that the majority of specimens used for the analysis of specimen type sensitivity were taken on the sixth day of illness or later, that is, on days when viral load was already low. If measured on the first days of illness, the probability to detect viral shedding may have been substantially higher in specimen types other than nasal wash and, thus, the difference between the sensitivities of various sampling methods may have been smaller.

Viral shedding, viral shedding profile, and symptom profile

The duration of viral shedding depends largely on 3 factors: 1) type of laboratory test (RT-PCR vs. viral culture), 2) age, and 3) treatment with antiviral medication. We detected viral RNA copies, on average, for 6.6 illness days, assuming that viral shedding starts with illness onset. This result is in agreement with that of Cao et al. (31), who found a median shedding duration of 6 days also while using polymerase chain reaction. We analyzed our data regarding the association of antiviral medication and age on the duration of viral shedding. Duration of shedding might be reduced by about 1.5 days when treatment is started ''on time'' (7.1 days vs. 5.7 days). Although this difference was not statistically significant (P ¼ 0.06), it must be taken into consideration that most of the cases who were treated within the first 3 illness days were index cases who tended to be more severely ill and started from a higher viral load. In our small data set, there was no apparent difference in age. In contrast, other studies on NIV have identified younger age as a risk factor for prolonged shedding (31, 32).

Our polymerase chain reaction data show that the detection limit is reached when samples contain less than 100 RNA copies/mL, whereas virus titration in cell culture starts to identify viruses in specimens with at least 10,000 RNA copies/mL. Our results suggest that viable (infectious) viruses cease to be present from the fifth illness day. This is in line with data from To et al. (32), while another study found that 24% of samples on illness day 7 still contained viable virus (33). This estimate compares well with data from seasonal influenza viruses, where the duration of shedding in untreated adult volunteers using viral culture was found to last for 4.8 days (5).

Comparison of the clinical course (expressed as symptom score) with viral load demonstrated that these 2 curves correlate well. This close correlation is well known from experimental studies in seasonal influenza (5) but has—to our knowledge—not yet been demonstrated for NIV. Nevertheless, individual data can, of course, differ markedly, which is shown by the fact that only 51% of the variance in viral load is ''explained'' by variations in symptom scores. We both observed substantial viral shedding while the patient showed mild clinical illness and also measured low or no viral loads despite typical influenza-like clinical presentation.

There are several limitations to this study. First, despite the proactive logistical setup of our study, we still had difficulties in collecting samples during the very first days of symptoms. Second, because of limited laboratory capacity, we sometimes had to stop taking further samples, although the last sample later turned out to be positive for NIV. Finally, although we had conducted a large number of prospective and labor-intensive field investigations, the study population was limited.

Our study provides systematically acquired observational data addressing several important questions on clinical manifestation, probability of transmission, duration of shedding and the possible effect of antiviral therapy, the preferred anatomical sampling site, the most sensitive specimen type, and the relation of shedding and clinical course in NIV. Overall, our study results do not seem to differ from what is known about seasonal influenza.

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Author affiliations: Department of Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany (Thorsten Suess, Udo Buchholz, Matthias an der Heiden, Walter Haas, Gérard Krause); Centre for Biological Safety, Division of Highly Pathogenic Microorganisms, Robert Koch Institute, Berlin, Germany (Susann Dupke, Roland Grunow); and National Reference Centre for Influenza, Robert Koch Institute, Berlin, Germany (Alla Heider, Barbara Biere, Brunhilde Schweiger).

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Tables and Figures

Table 1. Demographic, Clinical, and Treatment Characteristics Among Index Cases, Secondary Cases With/Without Antiviral Postexposure Prophylaxis, and Noncases, Germany, Summer 2009[a]

Characteristic	Index cases *(n=36)*		Secondary cases, under prophylaxis *(n=3)*		Secondary cases, not under prophylaxis (*n=12*)		Non-cases *(n=68)*	
	N	%	N	%	N	%	N	%
Male sex - n, (%)	18	(50)	1	(33)	3	(25)	31	(46)
Child (<14 yrs) – n, (%)	7	(19)	1	(33)	4	(33)	7	(10)
Clinical symptoms								
None- n, (%)	0	(0)	2	(67)	1	(8)	NA	NA
Fever > 38°C n, (%)	29	(81)	0	(0)	8	(67)	NA	NA
Cough- n, (%)	34	(94)	1	(33)	9	(75)	NA	NA
Sore throat-n, (%)	20	(56)	0	(0)	3	(25)	NA	NA
Headache/myalgia n, (%)	27[b]	(75)	0	(0)	4[b]	(33)	NA	NA
Influenza-like illness (Fever and [cough or sore throat]) - n, (%)	27	(75)	0	(0)	7	(58)	NA	NA
Hospitalisation- n, (%)	11	(31)	0	(0)	3	(25)	NA	NA
For isolation purposes- n, (%)	9	(25)	NA	NA	3	(25)	NA	NA
Due to severity of illness - n, (%)	2	(6)	NA	NA	0	(0)	NA	NA
Treatment with Oseltamivir- n, (%)	20	(56)	0	(0)	3	(25)	NA	NA
Within the first three days of illness - n, (%)	11	(31)	NA	NA	3	(25)	NA	NA
Prophylaxis with Oseltamivir - n, (%)	NA	NA	3	(100)	0	(0)	27	(40)
Chronic disease - n, (%)	6	(17)	0	(0)	3	(25)	9	(22)
Asthma- n, (%)	4	(11)	NA	NA	1	(8)	1	(1)
Cardiovascular disease- n, (%)	1	(3)	NA	NA	2	(17)	4	(6)
Other- n, (%)	1	(3)	NA	NA	0	(0)	4	(6)

Table 2. Number of data points, median and quartiles of symptom scores and log10 RNA copies/ml for each illness day.

	Symptom scores				Log10 of RNA copies/ml			
Illness day	**N**	**Median**	**Lower quartile**	**Upper quartile**	**N**	**Median**	**Lower quartile**	**Upper quartile**
1	40	2	1	4.5	3	4.81	2.00	8.33
2	38	4	2	5	6	5.22	4.42	6.55
3	38	3	1	5	11	4.64	3.54	5.81
4	40	2	1	4	20	4.49	3.73	5.44
5	39	2	1	3	27	3.84	2.80	4.37
6	38	1	1	2	30	3.57	2.00	4.43
7	38	1	1	2	32	3.28	2.00	4.26
8	34	1	0	2	31	2.00	2.00	3.21
9	31	0	0	1	33	2.00	2.00	2.00
10	30	0	0	1	34	2.00	2.00	2.00

Figure 1. Minimal duration of viral shedding in symptomatic cases (mean and 95% confidence interval), Germany, summer 2009. Minimal duration of shedding is shown for all symptomatic cases, as well as stratified by both therapy (timely antiviral therapy ¼ start of treatment within the first 3 days of illness; no (or no timely) antiviral therapy ¼ start of treatment later than the third day of illness or not at all) and age (children vs. adults). P values were calculated for comparisons between strata of therapy (timely therapy vs. no (or no timely) therapy, P ¼ 0.06) and age (children vs. adults, P ¼ 0.66) with Student's t test.

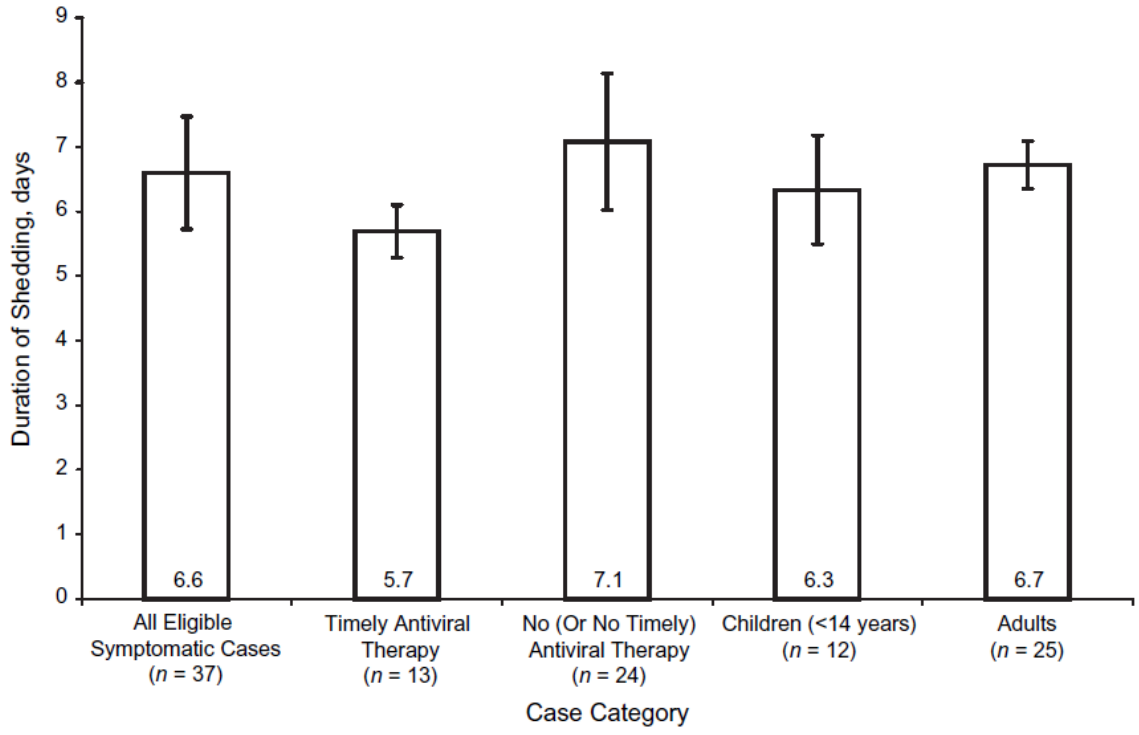

Figure 2. Comparison between clinical severity score (median and quartiles; dashed line) and viral shedding profile (median and quartiles of RNA copies/mL, log10; continuous line), Germany, summer 2009. Note that the y-axis starts at the value of 2 (polymerase chain reaction detection limit).