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Meta-Analysis

Time Lines of Infection and Disease in Human Influenza: A Review of Volunteer Challenge Studies

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The dynamics of viral shedding and symptoms following influenza virus infection are key factors when considering epidemic control measures. The authors reviewed published studies describing the course of influenza virus infection in placebo-treated and untreated volunteers challenged with wild-type influenza virus. A total of 56 different studies with 1,280 healthy participants were considered. Viral shedding increased sharply between 0.5 and 1 day after challenge and consistently peaked on day 2. The duration of viral shedding averaged over 375 participants was 4.80 days (95% confidence interval: 4.31, 5.29). The frequency of symptomatic infection was 66.9% (95% confidence interval: 58.3, 74.5). Fever was observed in 37.0% of A/H1N1, 40.6% of A/H3N2 ($p = 0.86$), and 7.5% of B infections ($p = 0.001$). The total symptoms scores increased on day 1 and peaked on day 3. Systemic symptoms peaked on day 2. No such data exist for children or elderly subjects, but epidemiologic studies suggest that the natural history might differ. The present analysis confirms prior expert opinion on the duration of viral shedding or the frequency of asymptomatic influenza infection, extends prior knowledge on the dynamics of viral shedding and symptoms, and provides original results on the frequency of respiratory symptoms or fever.

influenza, human; signs and symptoms; virus shedding

Abbreviations: CI, confidence interval; GEE, generalized estimating equations; HAI, hemagglutination inhibition; SD, standard deviation.

The threat of a human influenza pandemic has dramatically increased in recent years, and many countries have now developed pandemic preparedness plans following World Health Organization guidelines (1). Measures to reduce the spread of influenza within a given population, based on treatment or prophylaxis with antiviral medications, isolation, quarantine, or other social-distancing measures, are considered at various phases of the plans, as they might play a major role by reducing transmissibility. The effectiveness of these measures would depend greatly on the possibility of identifying infectious individuals and on how or when influenza virus is transmitted between individuals (2–5).

One critical question is whether the latent period overlaps the incubation period or, in other words, how onset of infectiousness overlaps onset of symptoms, if any (6). Another critical issue is the duration of infectiousness, which determines, among other things, the duration of treatment, prophylaxis, or isolation.

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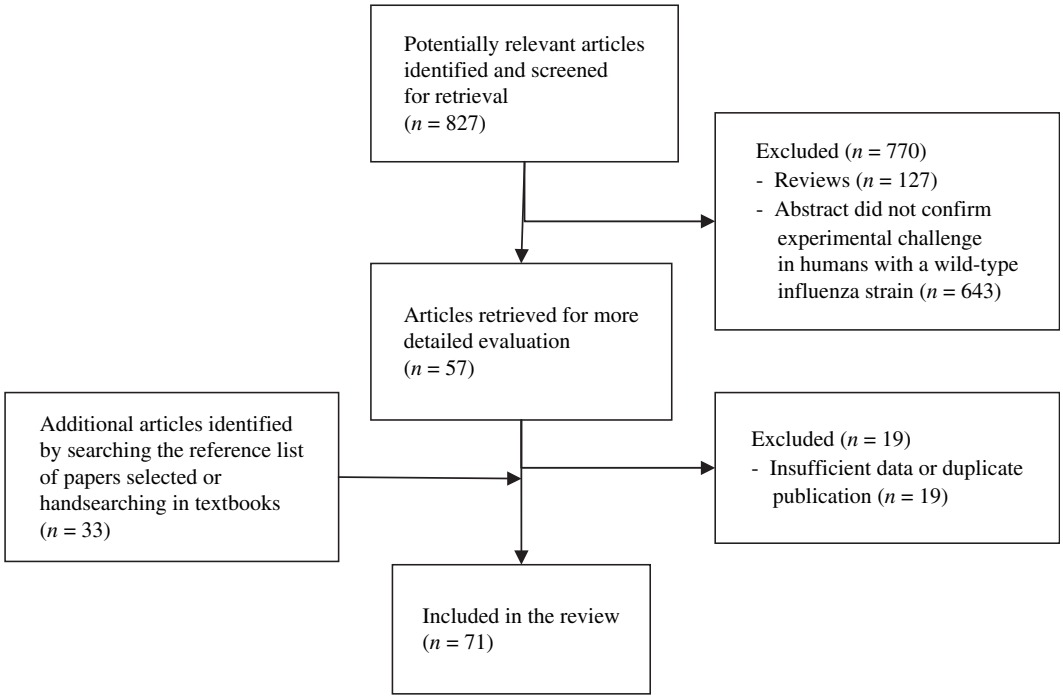

FIGURE 1. Identification of eligible articles.

Influenza infectiousness is usually equated to the presence of virus shedding. A recent report from the World Health Organization (7) concluded that influenza virus shedding can be detected 24–48 hours before clinical onset, and that it peaks during the first 24 hours of illness. Shedding usually lasts less than 5 days, but it may be higher and longer in children. The incubation period is reported to average 2 days (range: 1–4 days). These data are derived from expert opinions or may have involved observational and experimental studies without any attempt to use systematic review; they are not supported by high-quality evidence.

The frequency of asymptomatic infection is also a critical parameter for interventions involving contact tracing. Modeling studies used frequencies of between 30 percent and 50 percent (2–5, 8). However, these percentages come from pre- and post-influenza-season serologic studies, and they may therefore be subject to recall bias when individuals were asked whether they had had influenza-like illness during winter (9) or to classification bias due to lack of sensitivity of laboratory tests (10).

Experimental influenza virus infection of healthy volunteers provides a unique opportunity to describe the natural history, as 1) the date of infection is known with certainty, 2) shedding and symptoms are recorded prospectively, and 3) participants are usually selected with low pre-hemagglutination inhibition (HAI) antibody titers.

MATERIALS AND METHODS

Search strategy and identification of studies

A literature search was carried out using the PubMed database, with the keywords (''human influenza'' (text word) *or* ''influenza, human'' (MeSH terms)) *and* (''volunteer'' (all fields) *or* ''experimental'' (all fields) *or* ''deliberate infection'' (all fields) *or* ''shedding'' (all fields) *or* ''symptoms'' (all fields)). We limited our search to English-language papers published between 1965 and 2005. A total of 827 papers were selected (figure 1). We included any study with any design in which a subgroup of participants was challenged with a wild-type influenza virus and for which there was at least one type of outcome measure, that is, viral shedding or symptoms. We identified additional articles by searching the reference lists of articles. We also made a hand search in textbooks on influenza. We did not specifically consult world-leading specialists on influenza, but a bibliographic search on their names was performed. No attempt was done to retrieve primary data from the original studies. Two of us (F. C., M. L.) read all the studies retrieved in the search and applied the inclusion criteria. Differences were resolved by discussion and consensus.

Data abstraction

For each paper, we collected information on the year of publication, the number of study subgroups, the participants' characteristics, the number of participants, the type and subtypes of wild-type influenza virus used for challenge, the route of inoculation, the inoculated dose expressed in median tissue-culture infective doses, the duration of follow-up, and a summary of how clinical and virologic data were collected (refer to ''Supplementary Material''). (This information is posted on the *Journal*'s

website (http://aje.oxfordjournals.org/).) Note that one study can involve several subgroups of volunteers challenged with different influenza viruses and, conversely, several articles can describe the identical subgroup of volunteers but different outcomes (e.g., viral shedding or illness). Influenza virus infection was defined as a greater than fourfold rise in pre-HAI antibody titers or viral shedding (positive nasal wash cultures) at least 1 day after inoculation.

We extracted the following effect measures for each subgroup: proportion of infected individuals among those challenged, proportion of infected participants who shed virus (positive nasal wash on at least one occasion at least 1 day after inoculation), duration of viral shedding (time from inoculation to the first negative nasal wash with no subsequent positive washes), and proportion of infected participants who developed symptoms (any, systemic, or fever, respiratory, or nasal symptoms). We also described the dynamics of viral shedding, expressed in terms of the log-scale viral titer, and the dynamics of symptoms. Because various methods were used for scoring of symptoms, we normalized each study curve to its maximum clinical score of signs and symptoms. Summary curves were calculated as the weighted average of curves with weights equal to the number of individuals who were considered in each study curve. A further measure of shedding of interest is the first moment of the viral shedding curve. Recent work that estimated the generation time of influenza from household study data also showed that the resulting generation time distribution was quantitatively similar to viral shedding curves from experimental infection studies in human volunteers (4). This result is supportive of the hypothesis that infectiousness is proportional to viral shedding. Under this hypothesis, the average delay from a person's being infected to that individual's infecting other people, that is, the generation time (T_g), can be calculated as

$$T_g = \int_0^\infty tV(t)\mathrm{d}t \Big/ \int_0^\infty V(t)\mathrm{d}t,$$

where $V(t)$ is the absolute level of viral shedding at time t postinfection.

Statistical analysis

Summary effect measures were calculated. For summary means, a random-effect model was used, and the effect of covariates was assessed using the chi-square test for heterogeneity (11). For summary proportions, we used a binomial generalized estimating equations (GEE) model with an exchangeable correlation structure (12). The "clustered" effect was subgroup defined as the set of individuals challenged with the same influenza strain in each study. The GEE model gives population-averaged parameters, and it has been used for meta-analysis of rates (13, 14). The influence of a covariate on the effect measure was tested with the Wald chi-square test by introducing the covariate as a predictor of the effect measure in the GEE model. For all comparisons involving the type or subtype of influenza virus, A/H1N1 was chosen as the reference group. Statistical tests were two tailed, with a type I error risk of 5 percent.

RESULTS

Search results

Seventy-one papers describing 56 different studies, 79 different subgroups, and 1,280 different participants were considered. In all the studies, the participants were young adults aged between 18 and 40 or 50 years, except in one study (15), where the age ranged up to 65 years. A total of 199 (12 subgroups) participants had pre-HAI antibody titers to the challenge strain of at least 1/16 or were not selected according to their pre-HAI antibody titers, and these subjects were excluded from summary analyses of viral shedding or illness; 1,081 participants (67 subgroups) had a pre-HAI antibody titer that was considered unprotective (<1/16). A total of 532 volunteers were challenged with an A/H1N1 virus, 473 with an A/H3N2 virus, 86 with an A/H2N2 virus, and 189 with a type B virus. The routes of challenge were intranasal instillation in most studies. Throat sprays were also used in three studies (16–18), and aerosol inhalation was used in one study (19). The inoculum ranged between three and 7.2 $\log_{10}$ median tissue-culture infective doses. Most papers reported ethics committee approval or collection of written, informed consent from each participant. In almost all studies, participants were individually confined for 1 week. Most studies included daily follow-up with daily nasal washing and collection of clinical signs and symptoms. The follow-up period ranged from 3 days prior to inoculation to 14 days after inoculation.

Infection and viral shedding

The overall proportion of influenza virus infection in individuals with pre-HAI antibody titers of <1/16 was 88.2 percent (95 percent confidence interval (CI): 83.9, 91.4) (refer to Supplementary Material). Viral shedding was found in 93.1 percent of participants infected with A/H1N1, 92.5 percent with A/H3N2 ($p = 0.71$ vs. A/H1N1), and 83.9 percent with A/H2N2 virus ($p = 0.14$) and was lower in participants infected with a type B virus (81.5 percent, $p = 0.014$) (table 1).

Dynamics of viral shedding in infected volunteers

The dynamics of viral shedding are summarized in figure 2. The A/H1N1 and A/H3N2 curves showed a sharp increase during the first day following inoculation, and they reached their maximum values during the second day. Return to baseline values was obtained by day 8. The summary curves did not differ markedly according to influenza virus type or subtype, although A/H3N2 infections gave sustained high viral titers by comparison with A/H1N1.

On average, viral shedding was detected 1 day after inoculation. The distribution of the first observation of shedding was day 1 in 64 (83 percent) participants, day 2 in 11 (14 percent) participants, and day 3 in two (3 percent) participants (1.1 days, on average) (20). After challenge with A/H2N2 virus, four (40 percent) of the volunteers who shed the virus had culture-positive nasal washes by day 1 and 100 percent by day 3 (16). In two other studies, 14 (74 percent)

TABLE 1. Frequency of viral shedding (positive nasal wash on at least one occasion at least 1 day after inoculation) in healthy volunteers with low pre-hemagglutination inhibition antibody titers after experimental influenza virus infection*

Influenza virus types and subtypes	Subgroups (no.)	Infected participants (no.)	Viral shedding (no.)	Fixed-effect estimates (%)	95% confidence interval†	GEE estimates‡ (%)	95% confidence interval
A/H1N1	21	362	336	92.8	89.7, 95.3	93.1	88.5, 95.9
A/H3N2	18	228	210	92.1	87.8, 95.3	92.5	85.8, 96.1
A/H2N2	3	31	26	83.9	66.3, 94.6	84.3	64.9, 94.0
B	13	150	120	80.0	72.7, 86.1	81.5	67.0, 90.5
All	55	771	692	89.8	87.4, 91.8	90.0	85.6, 93.1

* p values for comparisons between influenza virus types or subtypes (A/H3N2 vs. A/H1N1: $p = 0.82$; A/H2N2 vs. A/H1N1: $p = 0.15$; B vs. A/H1N1: $p = 0.014$).

† Exact binomial confidence limits.

‡ GEE estimates, logistic generalized estimating equations model estimates.

(21) and 12 volunteers (86 percent) (22) had positive viral culture on the first day after inoculation. In the latter study, however, viral cultures became positive on days 4 and 5 in two volunteers.

When calculating the duration of viral shedding, we excluded three studies because of missing or inconsistent standard deviation values (23–25), and 23 study subgroups (375 participants) were considered. The mean duration of viral shedding was 4.80 days (95 percent CI: 4.31, 5.29) and did not differ according to the influenza virus types or subtypes: 4.50 days (95 percent CI: 3.71, 5.28) for type A/H1N1, 5.14 days (95 percent CI: 4.48, 5.80) for type A/H3N2 ($p = 0.22$, random-effect model), and 3.70 days (95 percent CI: 1.73, 5.66) for type B virus ($p = 0.46$).

Regarding the maximum duration of viral shedding, most volunteers had stopped shedding virus by day 6 or 7 (26, 27). However, longer durations are not rare: In one study subgroup, five (20 percent) participants still shed influenza B virus on day 8 after inoculation (15), and durations of A/H3N2 viral shedding ranging up to 9 days have been reported (28). In another study, three (30 percent) participants shed A/H2N2 virus until day 10 (16). In this latter case, however, the results were controversial: Volunteers were placed in isolation in groups of three, so that reinfection cannot be excluded.

The mean generation time calculated from viral shedding curves was 2.3 days (range: 1.5–2.7 days) for type A/H1N1, 3.1 days (range: 2.2–4.0 days) for type A/H3N2, and 3.4 days for type B virus. Across all studies, the mean generation time was 2.5 days.

Factors influencing viral shedding

There are few studies of factors associated with viral shedding. A dose-ranging study showed that the duration of shedding was proportional to the intranasal dose (29). Several studies suggested that volunteers were partially immune to the contemporary strain even though they had a low level of HAI antibodies in their serum before challenge (23, 30), thus explaining why some infected volunteers shed a small quantity of wild-type virus over a short period. This was supported by a significant difference in preexposure HAI antibody titers between participants with influenza virus infection who shed (average log titer = 0.7 (standard deviation (SD): 0.2)) and those who did not (average log titer = 1.5 (SD: 0.4)) (31).

Clinical illness

Any symptoms. Thirty-eight subgroups (522 infected individuals) were considered (table 2). The proportion of symptomatic infection (any symptoms) was 66.9 percent (95 percent CI: 58.3, 74.5). No significant difference was noted according to the virus type (refer to table 2 for p values) or the initial infectious dose ($p = 0.12$).

Respiratory symptoms. Upper respiratory symptoms, defined as nasal stuffiness, runny nose, sore throat, sneezing, hoarseness, ear pressure, or earache, were most frequent. The proportion of upper respiratory symptoms was 58.8 percent (95 percent CI: 45.5, 70.8) (table 3). A lower proportion was noted among participants infected with A/H3N2 virus compared with those infected with A/H1N1 virus, but the difference must be interpreted with care as only three small subgroups were considered for A/H3N2 infections.

Lower respiratory symptoms were defined as cough, breathing difficulty, and chest discomfort. Frequencies of lower respiratory symptoms were reported in six subgroups (four A/H1N1, one A/H3N2, one B; 119 infected participants). The proportion of lower respiratory symptoms was 21.0 percent (95 percent CI: 14.0, 30.3) and did not differ between virus types and subtypes or according to the inoculated dose.

Fever. Defined as a temperature of 100°F or 37.8°C or above, fever was reported in 34.9 percent (95 percent CI: 26.7, 44.2) of infected individuals (table 4). A lower proportion of fever in influenza B infection and a higher proportion in A/H2N2 infection were found as compared with A/H1N1 infections. A negative link was found between the dose and the proportion with fever (per $\log_{10}$ median tissue-culture infective dose increase: odds ratio = 0.56, 95 percent CI: 0.42, 0.73; $p < 0.001$).

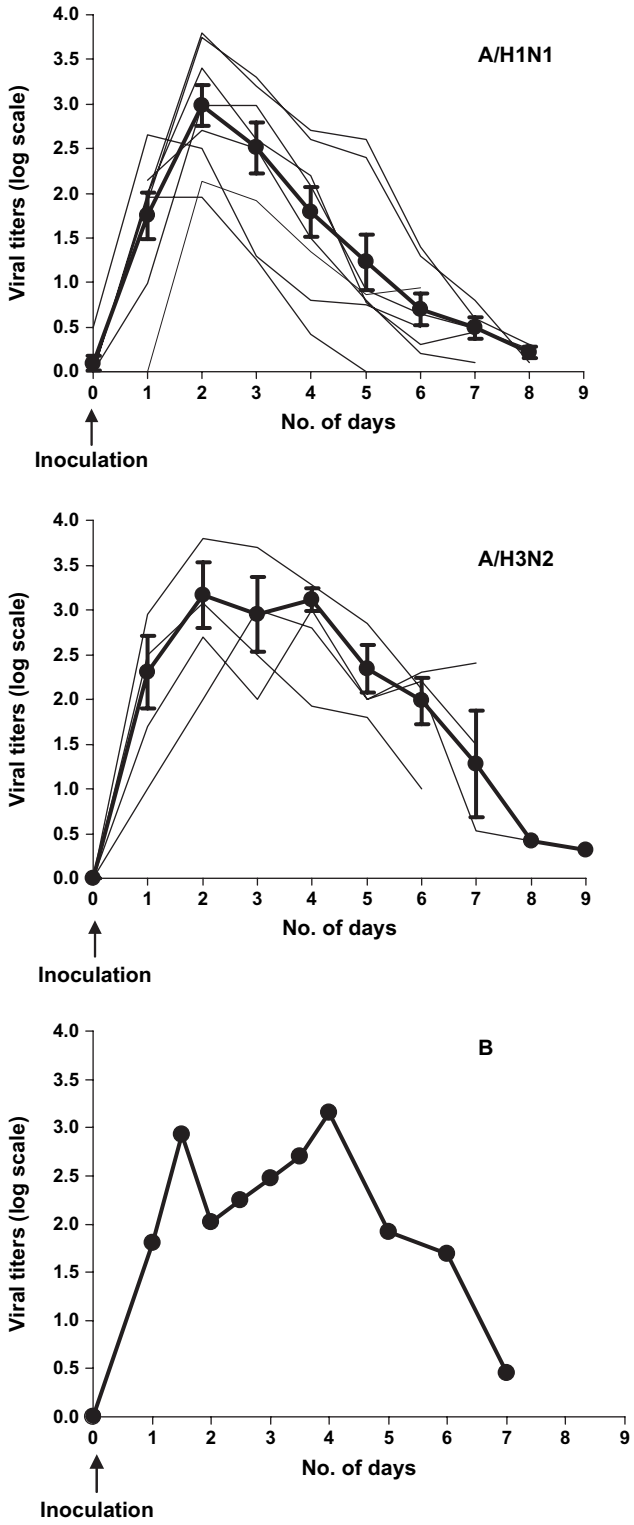

FIGURE 2. Summary curves of viral shedding in experimental influenza virus infection, according to the virus type or subtype. Eight curves (116 participants who shed influenza virus) for A/H1N1 subtype (21, 22, 24, 26, 32, 74–76) and four curves (41 participants) for A/H3N2 subtype (25, 28, 40, 45) were averaged, and one curve (eight participants) was plotted for the B type (24). Bold curves correspond to weighted averages of study curves (standard error).

Ear symptoms. Otologic manifestations were frequently reported. Ear pressure abnormalities were observed in 33–73 percent of 26 infected placebo recipients (32–35), and earache has been reported in 33–47 percent of adults (33). In one study, four participants (15 percent) developed signs of otitis media between days 5 and 7 after infection (36).

Dynamics of symptoms

An increase in the average total symptoms score was noted by day 1 after inoculation in A/H1N1 and A/H3N2 infections (figure 3). Total scores peaked by day 2 or day 3 and returned to baseline values by day 8. Individual incubation times were reported in 16 men who developed febrile illness after being inoculated with A/Bethesda/10/63 (H2N2) virus (37): Three men had an incubation period of 1 day, nine of 2 days, and four of 3 days (average: 2 days). In another subgroup, illness began an average of 1.7 days after challenge (38).

Systemic symptoms (fever, muscle aches, fatigue, headache) peaked earlier, by day 2 after inoculation, and resolved faster than respiratory or nasal symptoms (figure 4).

The mean duration of illness was rarely reported. The mean duration of illness was 4.4–5 days in 25 participants infected with A/H1N1 virus (34, 39), 3.7 days in seven participants challenged with A/H3N2 virus (40), 4.6 days in 13 participants infected with A/H2N2 virus (37), and 4.1 days in seven participants challenged with B virus (41).

Factors influencing clinical illness

The proportion and duration of illness were lower in the case of elevated pre-HAI titers. In two different studies with A/H3N2 virus challenge, the pooled proportions of illness (any symptoms) were 57 percent (20 of 34) in participants with pre-HAI titers $<1/12$, 52 percent (15 of 29) in participants with pre-HAI titers between 1/12 and 1/24, and 15 percent (two of 13) in participants with pre-HAI titers $>1/24$ (42, 43) ($p = 0.015$; Cochran-Armitage chi-square for a trend). The mean duration of illness was 4.4 days (SD: 1.8) in participants with pre-HAI titers of $\leq 1/8$ versus 1.0 day (SD: 1.4) in those with pre-HAI titers of $>1/8$ challenged with a wild-type A/H1N1 virus (34).

Relation between viral shedding and illness

Figure 5 describes the summary curves of viral shedding and total symptoms scores averaged over all influenza types and subtypes. The two curves showed similar shapes although viral shedding preceded illness by 1 day.

There is limited information on viral shedding in volunteers who did not develop clinical illness. In one study, three participants infected with a A/H3N2 virus who did not develop clinical illness excreted the virus, but the quantity of shedding was not available (44). We found only two studies using A/H3N2 viruses. The mean quantity of virus in nasal wash fluids from volunteers who shed virus and developed illness ($n = 11$) was from two $\log_{10}$ to three $\log_{10}$ times higher than in individuals who did not develop illness ($n = 14$), and a positive correlation was found between

TABLE 2. Proportion of volunteers who developed clinical illness after experimental influenza virus infection*

Influenza virus types and subtypes	Subgroups (no.)	Infected participants (no.)	Clinical illness (no.)	Fixed-effect estimates (%)	95% confidence interval†	GEE estimates‡ (%)	95% confidence interval
A/H1N1	11	228	158	69.3	62.9, 75.2	70.8	50.4, 85.2
A/H3N2	18	223	139	62.3	55.6, 68.7	64.5	54.6, 73.3
A/H2N2	3	31	24	77.4	55.4, 82.1	77.9	55.1, 91.0
B	6	40	25	62.5	45.8, 77.3	57.4	35.2, 76.9
All	38	522	346	66.3	62.1, 70.3	66.9	58.3, 74.5

* p values for comparisons between influenza virus types or subtypes (A/H3N2 vs. A/H1N1: $p = 0.68$; A/H2N2 vs. A/H1N1: $p = 0.57$; B vs. A/H1N1: $p = 0.29$).
† Exact binomial confidence limits.
‡ GEE estimates, logistic generalized estimating equations model estimates.

the mean quantity of virus per positive specimen and severity of illness (45, 46).

DISCUSSION

On the basis of a large review of experimental influenza virus infection of healthy volunteers, we found that average shedding of influenza virus increased during the first day after inoculation, consistently peaked on the second day, and lasted less than 5 days. One in three infected participants did not develop any clinical illness. Experimental influenza virus infection caused a mild disease, with mainly upper respiratory symptoms. Fever was observed in one third of participants, and lower respiratory symptoms, including cough, occurred in one in five participants.

A critical question is whether these findings are applicable to naturally acquired influenza virus infection. The answer will depend on three factors: the pathogenicity of the virus used to challenge volunteers, the host status as regards preexisting homo- and heterosubtypic immunity, and how the experimental challenge model accurately reflects acquisition of influenza in the real world.

It has been suggested that the viruses used in experimental studies were of moderate pathogenicity by comparison with wild-type seasonal influenza viruses (15, 24, 47, 48). It is noteworthy that two (8 percent) of 24 studies using A/H3N2 versus 19 (76 percent) of 25 studies using A/H1N1 were published after 1990 ($p < 0.001$), indicating a trend in the use of these respective influenza virus subtypes. This trend was likely due to the opinion that A/H3N2 infections gave more severe illnesses than did A/H1N1 infections, as higher rates of mortality or hospitalization have been reported with the A/H3N2 subtypes (49–51). However, we found no arguments supporting the idea that the challenge viruses were only moderately pathogenic. In particular, viral shedding and illness proportions did not differ according to the virus subtype. Two studies published within the 3 years after the emergence of the A/H3N2 influenza virus subtype used the pandemic strain to challenge seronegative volunteers (46, 52). They reported a proportion of ''any illness'' of 60 percent (15 of 25), consistent with the summary measure. The pathogenicity of influenza virus may also have been influenced by passage history through different viral media to produce an adequately sized pool for safety challenging. Influenza viruses are known to accumulate

TABLE 3. Proportion of volunteers who developed upper respiratory tract illness after experimental influenza virus infection*

Influenza virus types and subtypes	Subgroups (no.)	Infected participants (no.)	Upper respiratory tract illness (no.)	Fixed-effect estimates (%)	95% confidence interval†	GEE estimates‡ (%)	95% confidence interval
A/H1N1	8	144	103	71.7	63.5, 79.1	69.2	53.6, 81.3
A/H3N2	3	52	21	40.4	27.0, 54.9	38.4	17.2, 65.2
B	5	77	36	46.8	35.3, 58.5	56.3	31.3, 78.5
All	16	273	160	58.6	52.5, 64.5	58.8	45.5, 70.8

* p values for comparisons between influenza virus types or subtypes (A/H3N2 vs. A/H1N1: $p = 0.05$; B vs. A/H1N1: $p = 0.32$).
† Exact binomial confidence limits.
‡ GEE estimates, logistic generalized estimating equations model estimates.

TABLE 4. Proportion of volunteers who had fever (>100°F or >37.8°C) after experimental influenza virus infection*

Influenza virus types and subtypes	Subgroups (no.)	Infected participants (no.)	Fever (no.)	Fixed-effect estimates (%)	95% confidence interval†	GEE estimates‡ (%)	95% confidence interval
A/H1N1	15	285	88	30.9	25.6, 36.6	37.0	24.6, 51.3
A/H3N2	13	167	66	39.5	32.1, 47.4	40.6	30.9, 51.1
A/H2N2	1	10	10	100	69.2, 100	100§	69.2, 100§
B	7	101	7	7.0	2.8, 13.8	7.5	3.2, 16.9
All	36	563	171	30.4	26.6, 34.4	34.9	26.7, 44.2

* p values for comparisons between influenza virus types or subtypes (A/H3N2 vs. A/H1N1: $p = 0.86$; A/H2N2 vs. A/H1N1: $p = 0.001$; B vs. A/H1N1: $p = 0.001$).

† Exact binomial confidence limits.

‡ GEE estimates, logistic generalized estimating equations model estimates.

§ Fixed-effect estimate (too few subgroups for GEE estimate).

mutations in hemagglutinin during such passages. Hemagglutinin is an important antigen affecting the virulence of influenza viruses, and these mutations may have altered the antigenic characteristics of the viruses and their pathogenicity (53–55). Consequently, the apparent absence of differences across subtypes does not rule out the possibility that severity of illness was diminished in all subtypes in the experimental studies relative to natural acquisition of the same subtypes. In a study comparing naturally versus experimentally acquired influenza A (H3N2) infection, it was concluded that this factor may have explained in part the milder illness in experimentally infected volunteers (48). Comparability of the groups was, however, questionable in this study because the naturally infected group consisted of individuals who sought medical attention with acute febrile respiratory illness and thus were more severely ill and not representative of the clinical syndromes caused by influenza infection. In a prospective survey of households where an influenza-positive index patient was identified, fever or feverishness was found in only 16–32 percent of household contacts who developed an acute illness within 5 days of inclusion (56). These numbers are in line with our findings.

As regards preexisting homo- and heterosubtypic immunity, our review included more than 93 percent participants with $\leq$1/8 pre-HAI antibody titers, thus eliminating preexisting homotypic immunity (57). Heterosubtypic immunity is supported by both biologic evidence and epidemiologic theory (30, 58, 59), although its true mechanism is currently unknown. Heterosubtypic immunity, including immunity directed against other proteins of influenza virus, such as the neuraminidase or nucleoproteins, could have explained the mild disease in some volunteers but is also likely to preexist the emergence of an influenza strain in the adult population (60–62). We have no idea of how heterosubtypic immunity might affect the proportion or dynamics of shedding and illness, but we believe that findings in the healthy adult population with an unknown degree of heterosubtypic immunity and a low level of homotypic immunity would apply to most influenza epidemics.

As regards how the experimental challenge model mimics reality, the reviewed studies have the potential limitation that most participants were inoculated using intranasal instillations. Although large droplets are thought to be the main mode of influenza transmission (7, 63–65), direct contact with secretions or aerosols can play a role in natural infections (66). We found only one study using aerosol inhalation (19). In 11 adult volunteers with low neutralizing antibody titers who were challenged with A/H2N2 virus, six were infected and typical clinical influenza occurred in four. On the basis of these results and those of older studies, it was stated that participants infected by intranasal drop inoculation had a milder disease, a longer incubation time, and a lower proportion of involvement of the lower respiratory tract than those infected by aerosol inoculation (66). Because of the low number of subjects, these statements are not supported by strong statistical evidence, but we cannot exclude that the symptoms initiated by intranasal inoculation may not cover the full spectrum of symptoms seen in natural infections. Finally, in our review, the proportions of symptomatic infection were consistent with the proportions observed in community studies of naturally acquired influenza virus infection, which included all possible routes of transmission (9, 10).

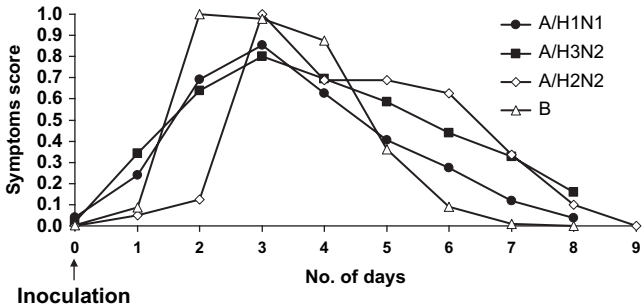

FIGURE 3. Summary curves of total symptoms scores in experimental influenza virus infection, according to the virus type or subtype. Seven curves (134 infected participants) for A/H1N1 subtype (21, 22, 26, 76, 77) and eight curves (68 participants) for A/H3N2 subtype (18, 25, 40, 43, 45, 48, 78) were averaged, and one curve (11 participants) for A/H2N2 subtype (16) and one curve (15 participants) for B type (17) were plotted. A total score of 1 corresponds to the maximum reported score value (refer to Materials and Methods).

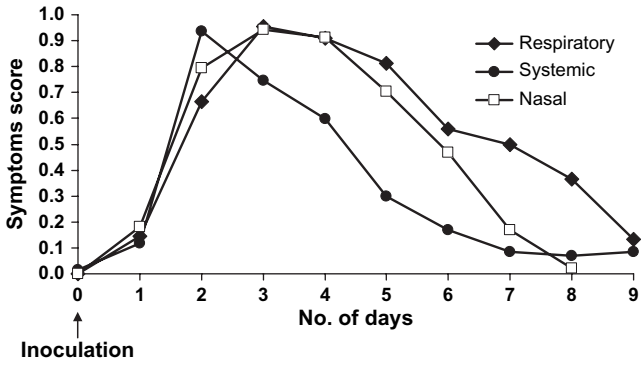

FIGURE 4. Summary curves of systemic symptoms (fever, muscle aches, fatigue, headache), respiratory symptoms, or nasal symptoms scores. Seven curves (159 infected participants) were considered for the systemic scores (20, 34, 74, 79–82), five curves (132 participants) for the nasal scores (20, 34, 79–81), and two curves (28 participants) for the respiratory scores (28, 75). A score of 1 corresponds to the maximum reported score value (refer to Materials and Methods).

The duration of shedding may have been influenced by the choice of sampling site or the sensitivity of the virologic methods (67), but the experimental conditions would have limited this type of bias. We used summary effect measures and had no access to individual data. This prevented us from describing the variability of several possibly important parameters. Finally, several reports suggest that, compared with otherwise healthy adults, children can shed virus earlier before the illness begins and for a longer period once the illness starts (68–70). In one report, presymptomatic shedding was described up to 6 days before clinical onset (68). However, the findings must be interpreted with care, as these data were collected retrospectively, and no children with positive viral shedding preceding clinical onset were identified in the prospective part of the study. In another report, in 63 hospitalized children, the duration of positive virus isolation was 6.8 days (SD: 1.7) in influenza A and 6.2 days (SD: 1.3) in influenza B infections (70). We are not aware of such data in the elderly. Cytotoxic T-lymphocyte activity is responsible for viral clearance and recovery from infection (71). Cytotoxic T-lymphocyte activity declines with age (72), and we can suspect that viral shedding would persist longer in the elderly as shown in immunocompromised individuals (73). The impaired cellular response would decrease the frequency of symptoms associated with production of cytokines (e.g., fever) (74) and would increase the risk of complications.

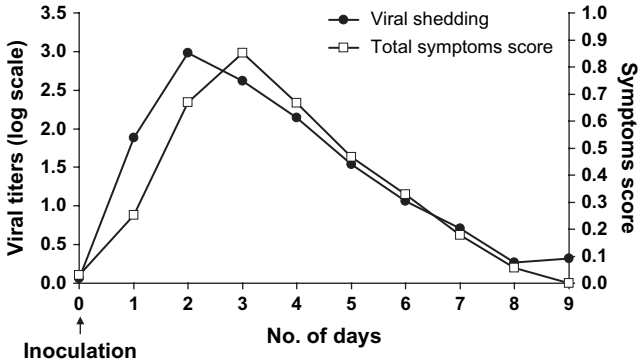

FIGURE 5. Summary curves of viral shedding and total symptoms scores in experimental influenza virus infection. Thirteen curves were used for viral shedding (refer to figure 2 legend), and 17 curves were used for total symptoms scores (refer to figure 3 legend).

We found a striking negative link between the inoculated dose and the proportion of fever. We have no explanation for this result. Particularly, the apparent correlation was not due to a difference of influenza subtypes or a time trend.

Epidemiologically, one is as much interested in how the infectiousness of an infected individual varies over time as the time someone sheds virus at above a detectable level. This is best captured by the generation time of an influenza epidemic. Our analysis indicates that the generation time of influenza may be as short as 2.5 days on average (range: 1.5–4.0 days). This value is consistent with the value of 2.6 days estimated from epidemiologic data (4) and substantially shorter than other epidemiologic modeling studies have assumed (2, 5).

To conclude, our analysis confirms prior expert opinion on the duration of viral shedding or the frequency of asymptomatic influenza infection, extends prior knowledge on the dynamics of viral shedding and symptoms, and provides original results on the frequency of respiratory symptoms or fever. Optimistically, viral shedding, the surrogate marker of infectiousness, was of moderate duration, and its dynamics largely overlapped those of systemic symptoms, thus (in theory) permitting efficient isolation of infectious individuals. Pessimistically, viral shedding peaked rapidly, infections were rarely ''typical,'' and symptoms or signs widely used for influenza case definitions (e.g., fever or cough) would be unreliable for identifying infectious individuals.

Urgent research needs include the role of heterosubtypic immunity and the natural history of influenza virus infection in children and the elderly.

Editor's note: *References 83–111 are cited in the Web-only Supplementary Material posted on the* Journal*'s website (http://aje.oxfordjournals.org/).*

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