Clinical Infectious Diseases

MAJOR ARTICLE

Association Between the Respiratory Microbiome and Susceptibility to Influenza Virus Infection

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Background. Previous studies suggest that the nose/throat microbiome may play an important role in shaping host immunity and modifying the risk of respiratory infection. Our aim is to quantify the association between the nose/throat microbiome and susceptibility to influenza virus infection.

Methods. In this household transmission study, index cases with confirmed influenza virus infection and their household contacts were followed for 9–12 days to identify secondary influenza infections. Respiratory swabs were collected at enrollment to identify and quantify bacterial species via high-performance sequencing. Data were analyzed by an individual hazard-based transmission model that was adjusted for age, vaccination, and household size.

Results. We recruited 115 index cases with influenza A(H3N2) or B infection and 436 household contacts. We estimated that a 10-fold increase in the abundance in *Streptococcus* spp. and *Prevotella salivae* was associated with 48% (95% credible interval [CrI], 9–69%) and 25% (95% CrI, 0.5–42%) lower susceptibility to influenza A(H3N2) infection, respectively. In contrast, for influenza B infection, a 10-fold increase in the abundance in *Streptococcus vestibularis* and *Prevotella* spp. was associated with 63% (95% CrI, 17–83%) lower and 83% (95% CrI, 15–210%) higher susceptibility, respectively.

Conclusions. Susceptibility to influenza infection is associated with the nose/throat microbiome at the time of exposure. The effects of oligotypes on susceptibility differ between influenza A(H3N2) and B viruses. Our results suggest that microbiome may be a useful predictor of susceptibility, with the implication that microbiome could be modulated to reduce influenza infection risk, should these associations be causal.

Keywords. influenza; microbiome; susceptibility; transmission.

Influenza causes an estimated 3 to 5 million severe illnesses and 400 000 deaths annually [1, 2]. Vaccination is currently the most effective strategy for controlling influenza transmission, but vaccine effectiveness has been suboptimal in recent seasons mostly due to vaccine–virus mismatch [3]. Moreover, vaccine coverage is typically low in low- and middle-income countries [4]. To develop complementary influenza-prevention strategies, it is important to identify host determinants of influenza susceptibility.

One potential prevention strategy is to manipulate the host nasal/throat microbiome. Both animal models and human studies suggest that the respiratory microbiome in the nose/throat may affect host immunity and modify susceptibility to viral respiratory infections, including influenza [5–9]. However, much remains to be learned, including associations between different microbiota compositions and risk of different viral infections, and the possibility of manipulating the microbiome to prevent infection.

As one of the major venues for influenza transmission, the household is an ideal setting to examine the relationship between biological characteristics of hosts, including the respiratory microbiome, and their risk of influenza infection [10, 11]. In a typical household transmission study, exposed household contacts are closely monitored for influenza infection intensively in the approximately 2 weeks following illness onset in an index case, a period when they are highly infectious [11]. The household secondary attack rate, defined as the risk of infection for household members living with an index case, of seasonal influenza ranges from 10% to 20% [12]. We conducted a household influenza transmission study in Managua, Nicaragua, during 2012–2014 and identified an association between influenza transmission and a microbiome community state type. However, that analysis is based on simple regression methods and the results are not specific to influenza type/subtypes [13]. Another analysis based on the same data found that higher bacterial community diversity prior to infection was associated

Received 7 June 2019; editorial decision 23 September 2019; accepted 26 September 2019; published online September 28, 2019.

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Clinical Infectious Diseases® 2020;71(5):1195–203

DOI: 10.1093/cid/ciz968

with longer shedding duration and earlier time to infection [14]. This indicates that the role of the microbiome in influenza transmission could be critical.

Here we present a more thorough analysis of the same data using a transmission model that is capable of quantifying the type/subtype-specific relationship between the nasal/throat microbiota and susceptibility to influenza A(H3N2) and B virus infection. This model accounts for transmission dynamics, including timing of infections, community infection risk, and household transmission chains, providing a more comprehensive picture of the role of the respiratory microbiome on influenza risk.

METHODS

Study Subjects

Detailed methods for the household influenza transmission study have been published [15]. Briefly, index influenza cases were identified at the Health Center Sócrates Flores Vivas, a primary care facility. Index cases were enrolled if (1) they experienced influenza-like illness, defined as fever or feverishness with cough, sore throat, or rhinorrhea, with symptom onset at 48 hours or earlier; (2) they were positive for influenza by rapid antigen test; (3) they had no other household members with influenza symptoms in the previous 2 weeks; and (4) they were living with 1 or more household member. Following enrollment, we conducted a household visit to collect initial respiratory samples and obtain demographic and symptom information. We visited each household up to 4 additional times, every 2–3 days, to collect respiratory samples and daily symptom information. This study was approved by the institutional review boards at the Nicaraguan Ministry of Health and the University of Michigan. Written consent to participate or parental permission was obtained for all participants. Verbal assent was obtained for children aged 6 years or older.

Laboratory Methods

Combined nasal and throat swabs were stored at 4–8°C in viral transport medium and transported to the National Virology Laboratory within 12 hours of collection. Samples were tested for influenza virus types (A and B) and subtypes (H1N1pdm and H3N2) by real-time reverse transcriptase–polymerase chain reaction (RT-PCR) on an Applied Biosystems 7500 Fast PCR platform following validated protocols from the US Centers for Disease Control and Prevention. Sample aliquots were stored at −70°C for microbiota characterization.

Microbiota Characterization

We characterized the microbiota of swabs collected at the first home visit. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) and an additional solution consisting of cell lysis solution (Promega), lysozyme, mutanolysin, RNase A, and lysostaphin (Sigma-Aldrich).

The V4 hypervariable region of the 16S rRNA gene was sequenced using Illumina MiSeq V2 chemistry 2x250 (Illumina) and a validated dual-indexing method [13]. Following alignment and quality filtering in mothur v1.38.1 (www.mothur.org/wiki/MiSeq_SOP, accessed 18 November 2016) reads were partitioned into unique taxonomic units (oligotypes) using Minimum Entropy Decomposition with default parameters (-M: 13779.0, -V: 3 nt). Representative sequences of oligotypes were classified using the Human Oral Microbiome Database v14.51 [16] and blastn v2.2.23 [17]. Taxonomic classifications with ≥Taxidentity (≥98% identity) were kept.

After excluding any sample with fewer than 1000 total reads, samples were assigned to 5 bacterial community types using Dirichlet multinomial mixture models [18] and the DirichletMultinomial v1.16.0 R package [19]. The final number of community types was selected based on fit of the negative log models and statistical power in downstream analysis. The community type for an individual was assigned as missing when his/her maximum posterior probabilities for community types were less than 0.9 [18].

Statistical Models

Our analysis focused on RT-PCR–confirmed influenza A(H3N2) and B infection. There were only 16 index cases with influenza A(H1N1)pdm infection, which were insufficient to conduct robust estimation. We used an individual-based hazard household transmission model [20, 21] to characterize transmission in households, involving risks of infection from outside the household ("community infections") or from infected household members ("secondary or tertiary infections") to estimate the association between the nose/throat microbiome at enrollment and influenza susceptibility. This relaxed an assumption that all household contacts were infected by their index cases, which is commonly made in regression-type analyses (Supplementary Information, Section 1).

In this model, the infection risk of each contact depended on the infectivity of other infected household members (determined by the time since symptom onset) and a daily infection risk from the community. Serial intervals, defined as the time between 2 consecutive cases, were assumed to follow a discretized Weibull distribution. In the transmission analyses, we adjusted for age (≤18 years vs >18 years), vaccination status (seasonal trivalent inactivated influenza vaccine prior to that influenza season), and household size (<4 people vs ≥5 people).

We used 2 different measures of the nose/throat microbiome in the model. First, we considered the community types identified as described above. Second, we used oligotypes that account for more than 50% of the difference between community types [22]. For the second measure, we conducted the analyses in 2 steps because the number of oligotypes is large. In step 1, we used the model to fit each oligotype separately to explore oligotypes potentially associated with susceptibility, defined as

Table 1. Characteristics of Index Case Patients With Influenza A(H3N2) or B Virus Infection and Their Household Contacts

Characteristics	Influenza A(H3N2)	Influenza B
Index cases		
No. of index cases	76	39
Age, years		
0–5	47 (62)	16 (41)
6–17	24 (32)	22 (56)
>17	5 (7)	1 (3)
Male	40 (53)	22 (56)
Prior vaccination	2 (3)	3 (8)
Oseltamivir treatment	67 (88)	37 (95)
Microbial community type		
1	23 (30)	8 (21)
2	17 (22)	6 (15)
3	10 (13)	5 (13)
4	10 (13)	8 (21)
5	15 (20)	10 (26)
Missing	1 (1)	2 (5)
Number of household contacts		
1–3	45 (59)	25 (64)
4–5	17 (22)	6 (15)
≥6	14 (18)	8 (21)
Number of secondary cases in household		
0	51 (67)	18 (46)
1	15 (20)	14 (36)
2	5 (7)	5 (13)
≥3	5 (7)	2 (5)
Symptom profile		
Fever	76 (100)	39 (100)
Rhinorrhea	74 (97)	37 (95)
Sore throat	37 (49)	15 (38)
Cough	72 (95)	36 (92)
ILI[a]	72 (95)	37 (95)
Household contacts		
No. of contacts	286	150
Age, years		
0–5	33 (12)	19 (13)
6–17	88 (31)	45 (30)
>17	165 (58)	86 (57)
Male	106 (37)	58 (39)
Prior vaccination	6 (2)	18 (12)
Bacterial community type		
1	72 (25)	36 (24)
2	57 (20)	38 (25)
3	69 (24)	30 (20)
4	41 (14)	23 (15)
5	36 (13)	14 (9)
Missing	11 (4)	9 (6)
Household contacts with RT-PCR–confirmed infection		
Overall	43/286 (15)	32/150 (21)
Age, years		
0–5	13/33 (39)	8/19 (42)
6–17	17/88 (19)	16/45 (36)
>17	13/165 (8)	8/86 (9)
Symptom profile		
Fever	19/43 (44)	22/32 (69)
Rhinorrhea	24/43 (56)	20/32 (62)
Sore throat	15/43 (35)	14/32 (44)
Cough	26/43 (60)	18/32 (56)
ILI[a]	17/43 (40)	15/32 (47)

Data are presented as n (%) or n/N (%) unless otherwise indicated.
Abbreviations: ILI, influenza-like illness; RT-PCR, reverse transcriptase–polymerase chain reaction.
[a]Fever with sore throat or cough.

a 90% credible interval of the risk ratio excluding 1. In step 2, we used the transmission model to fit all oligotypes selected by step 1 together.

Inference

Model fitting was conducted in a Bayesian framework. Association is defined as a risk ratio with a 95% credible interval excluding 1. To account for missing data in community type, we constructed a Markov Chain Monte Carlo algorithm [23] that permits the sampling of missing community types. Hence, model parameters and missing community types were estimated jointly (Supplementary Information, Section 2). We conducted simulation to evaluate our model adequacy (Supplementary Information, Section 3). Statistical analyses were conducted using R version 3.2.1. Data and code availability are summarized in Supplementary Information, Section 4.

RESULTS

Study Participants

We recruited 76 individuals with PCR-confirmed influenza A(H3N2) virus and 39 individuals with PCR-confirmed influenza B virus infection together with 286 and 150 household contacts, respectively, between August 2012 and November 2014 (Table 1). The proportion of households with at least 1 secondary case for influenza A(H3N2) index cases was lower than that for influenza B index cases ($P = .048$). Other characteristics of index cases were similar between the households affected by influenza A(H3N2) and influenza B. The proportion of vaccinated household contacts of influenza A(H3N2) index cases was lower than that of influenza B index cases ($P < .0001$). Other characteristics for household contacts were similar between influenza A(H3N2) and B households. The observed risks of PCR-confirmed infection in household contacts were 15.0% (43/286; 95% confidence interval [CI], 11.1–19.7%) and 21.3% (32/150; 95% CI, 15.1–28.8%) for influenza A(H3N2) and B, respectively.

The characteristics of household contacts stratified by influenza virus type of index cases and baseline bacterial community type are presented in Table 2. The proportion of children among household contacts with community type 4 was lower than those with other community types, regardless of influenza

Table 2. Characteristics of Household Contacts by Bacterial Community Type

Characteristics	Bacterial Community Type					
	1	2	3	4	5	Missing
Influenza A(H3N2)						
No. of contacts	72	57	69	41	36	11
Age, years						
0–5	6/72 (8)	4/57 (7)	5/69 (7)	1/41 (2)	14/36 (39)	3/11 (27)
6–17	30/72 (42)	21/57 (37)	15/69 (22)	10/41 (24)	7/36 (19)	5/11 (45)
>17	36/72 (50)	32/57 (56)	49/69 (71)	30/41 (73)	15/36 (42)	3/11 (27)
Male	29/72 (40)	17/57 (30)	21/69 (30)	18/41 (44)	16/36 (44)	5/11 (45)
Prior vaccination	0/72 (0)	2/57 (4)	2/69 (3)	0/41 (0)	1/36 (3)	1/11 (9)
Infection confirmed						
Overall	11/72 (15)	8/57 (14)	13/69 (19)	1/41 (2)	8/36 (22)	2/11 (18)
Age 0–5 years	3/6 (50)	0/4 (0)	3/5 (60)	0/1 (0)	5/14 (36)	2/3 (67)
Age 6–17 years	6/30 (20)	5/21 (24)	3/15 (20)	1/10 (10)	2/7 (29)	0/5 (0)
Age >17 years	2/36 (6)	3/32 (9)	7/49 (14)	0/30 (0)	1/15 (7)	0/3 (0)
Symptom profile						
Fever	5/11 (45)	4/8 (50)	4/13 (31)	1/1 (100)	5/8 (62)	0/2 (0)
Rhinorrhea	4/11 (36)	7/8 (88)	6/13 (46)	1/1 (100)	6/8 (75)	0/2 (0)
Sore throat	4/11 (36)	4/8 (50)	5/13 (38)	0/1 (0)	2/8 (25)	0/2 (0)
Cough	5/11 (45)	7/8 (88)	8/13 (62)	1/1 (100)	5/8 (62)	0/2 (0)
ILI[a]	4/11 (36)	4/8 (50)	4/13 (31)	1/1 (100)	4/8 (50)	0/2 (0)
Influenza B						
No. of contacts	36	38	30	23	14	9
Age, years						
0–5	2/36 (6)	8/38 (21)	0/30 (0)	0/23 (0)	5/14 (36)	4/9 (44)
6–17	10/36 (28)	12/38 (32)	12/30 (40)	5/23 (22)	4/14 (29)	2/9 (22)
>17	24/36 (67)	18/38 (47)	18/30 (60)	18/23 (78)	5/14 (36)	3/9 (33)
Male	13/36 (36)	14/37 (37)	9/30 (30)	12/23 (52)	6/14 (43)	4/9 (44)
Prior vaccination	7/36 (19)	3/37 (8)	5/30 (17)	1/23 (4)	2/14 (14)	0/9 (0)
Infection confirmed						
Overall	10/36 (28)	8/38 (21)	5/30 (17)	4/23 (17)	1/14 (7)	4/9 (44)
Age 0–5 years	2/2 (100)	4/8 (50)	NA	NA	0/5 (0)	2/4 (50)
Age 6–17 years	3/10 (30)	3/12 (25)	5/12 (42)	3/5 (60)	1/4 (25)	1/2 (50)
Age >17 years	5/24 (21)	1/18 (6)	0/18 (0)	1/18 (6)	0/5 (0)	1/3 (33)
Symptom profile						
Fever	5/10 (50)	4/8 (50)	4/5 (80)	4/4 (100)	1/1 (100)	4/4 (100)
Rhinorrhea	7/10 (70)	5/8 (62)	3/5 (60)	2/4 (50)	0/1 (0)	3/4 (75)
Sore throat	3/10 (30)	4/8 (50)	3/5 (60)	3/4 (75)	1/1 (100)	0/4 (0)
Cough	5/10 (50)	6/8 (75)	3/5 (60)	2/4 (50)	1/1 (100)	1/4 (25)
ILI[a]	3/10 (30)	4/8 (50)	3/5 (60)	3/4 (75)	1/1 (100)	1/4 (25)

Data are presented as n/N (%) unless otherwise indicated.
Abbreviations: ILI, influenza-like illness; NA, not applicable.
[a]Fever with sore throat or cough.

virus type ($P < .01$ for influenza A(H3N2), $P = .046$ for influenza B). The observed risk of influenza A(H3N2) virus infection among household contacts with community type 4 was lower (2%; 95% CI, 0–13%) than with other community types (14–22%). Increased and decreased risks of influenza B infection among household contacts were observed for community type 1 (28%; 95% CI, 14–45%) and type 5 (7%; 95% CI, 0–34%), respectively, compared with other community types (17–28%). The distributions of oligotypes in each of the baseline community types among household contacts, stratified by influenza type of the index case, are summarized in Supplementary Table 1. The range of the abundance of those oligotypes is shown in Supplementary Figure 1.

Association Between Community Type and Influenza Virus Infection Susceptibility

We fitted our transmission model to estimate the association between community type and susceptibility to influenza, while accounting for missing community types (Figure 1A, Supplementary Tables 2 and 3). Simulations suggested that our model provided reasonable fit to the data (Supplementary Figure 2). After adjusting for age groups, vaccination status, and

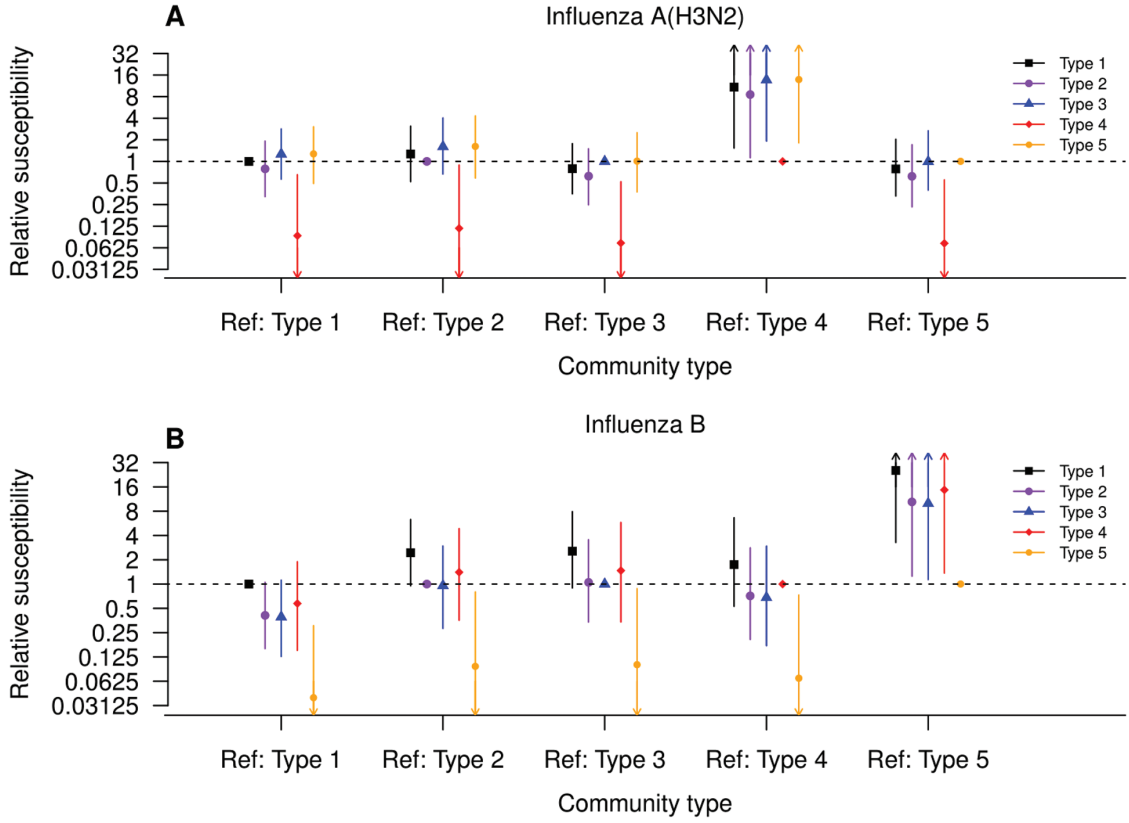

Figure 1. *A* and *B*, Association between bacterial community types in household contacts and susceptibility to PCR-confirmed influenza infection, by influenza type estimated by the household transmission model. Squares (black), larger circles (purple), triangles (blue), diamonds (red), and smaller circles (orange) represent community types 1 to 5, respectively. Abbreviations: PCR, polymerase chain reaction; Ref, reference group.

household size, community type 4 was associated with lower susceptibility to influenza A(H3N2) infection, with risk ratios of 0.07 to 0.12 in reference to the other 4 community types. Community type 5 (shown in yellow; Figure 1B) was associated with lower susceptibility to influenza B infection, with risk ratios of 0.04 to 0.10, compared with the other 4 community types. As a sensitivity analysis, we refitted the model with symptomatic influenza infection and the results were similar (Supplementary Table 4).

Oligotype Contribution to Susceptibility

We explored which oligotypes that accounted for more than 50% of the difference between community types contribute to susceptibility. Step 1 transmission analyses were models fitted for each oligotype, adjusted for age, vaccination, and household size (Figure 2A, Supplementary Table 5) We found that *Streptococcus* species (spp.) (*dentisani*, *mitis*, *oralis*, *infantis*, *tigurinus*, *lactarius*, *peroris*, *pneumoniae*) and *Prevotella salivae* were potentially associated with lower susceptibility to influenza A(H3N2) infection. Step 2 transmission analyses were models fitted with these oligotypes together, adjusted for the same variables in the first step (Figure 2B). We estimated that a 10-fold increase in the abundance in *Streptococcus* spp. (*dentisani*, *mitis*, *oralis*, *infantis*, *tigurinus*, *lactarius*, *peroris*, *pneumoniae*) and *Prevotella salivae* was associated with 48% (95% credible interval [CrI], 9–69%) and 25% (95% CrI, 0.5–42%) lower susceptibility to influenza A(H3N2) infection. *Streptococcus* spp. (*dentisani*, *mitis*, *oralis*, *infantis*, *tigurinus*, *lactarius*, *peroris*, *pneumoniae*) was the most abundant oligotype in community type 4, suggesting that the lower susceptibility to influenza A(H3N2) infection with community type 4 may be possibly explained by this oligotype.

In contrast, we found 5 oligotypes that were potentially associated with susceptibility to influenza B infection in step 1 analyses (Figure 2A, Supplementary Table 3). Based on the multivariate model including these 5 oligotypes, we found that a 10-fold increase in the abundance of *Streptococcus vestibularis*, *salivarius*, and *gordonii* spp. was associated with 63% (95% CrI: 17%, 83%) lower susceptibility, while a 10-fold increase in the abundance of *Prevotella* spp. (*veroralis*, *fusca*, *histicola*, *scopos*, *melaninogenica*) was associated with 83% (95% CrI, 15–210%) higher susceptibility to influenza B infection. Different from community types 1–4, *Streptococcus vestibularis*, *salivarius*, and *gordonii* spp. and *Prevotella* spp. *veroralis*, *fusca*, *histicola*, *scopos*, and *melaninogenica* were the most and least abundant oligotypes in community type 5, suggesting that the lower susceptibility to influenza B infection among individuals with community type 5 may be mostly explained by these 2 oligotypes.

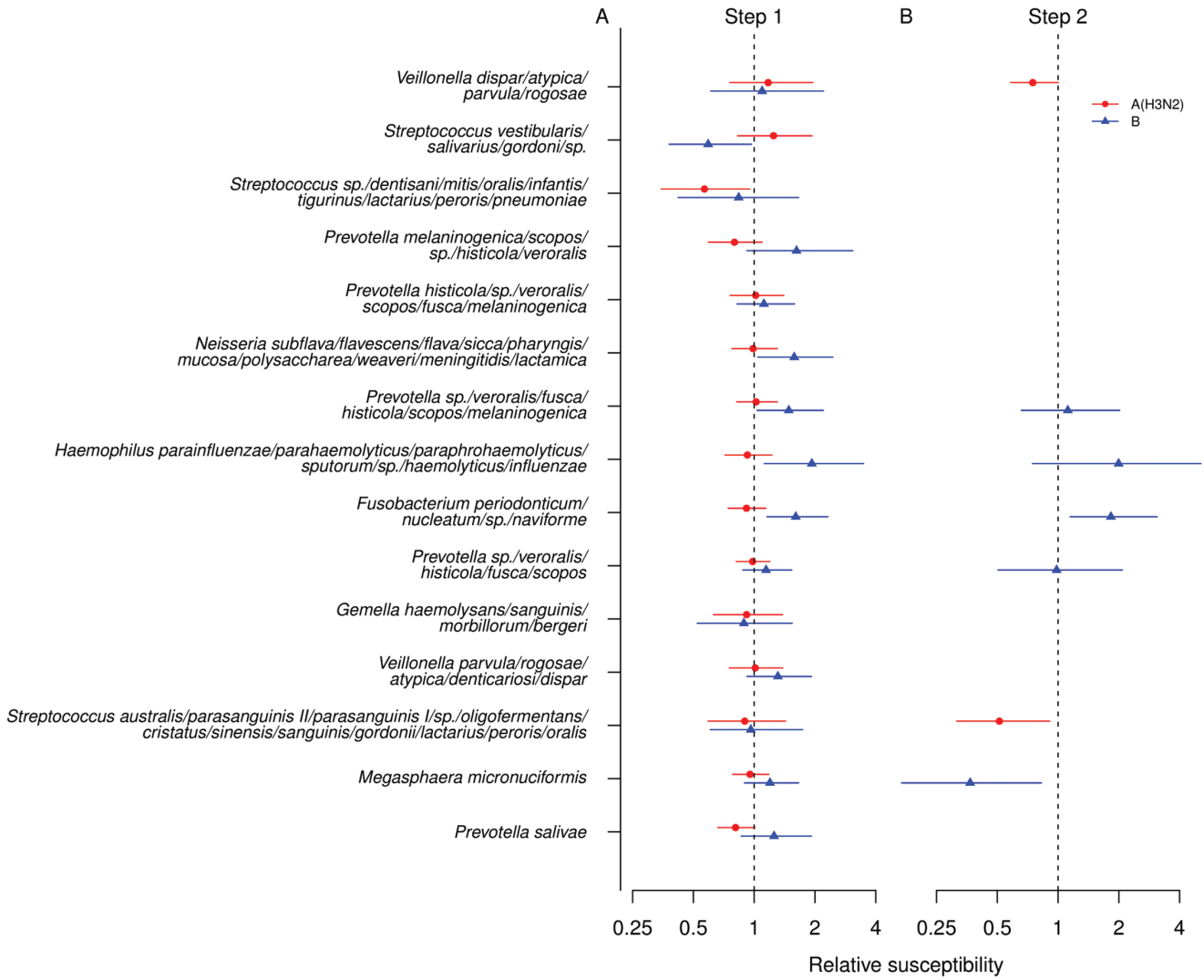

Figure 2. Association between bacterial oligotype and susceptibility to PCR-confirmed infection for household contacts estimated by household transmission model. Circles (red) and triangles (blue) indicate PCR-confirmed influenza A(H3N2) and B virus infection, respectively. *A*, The points and lines represented the point estimate and 90% credible intervals of the association between oligotypes and susceptibility estimated in separate models in step 1. *B*, The points and lines represented the point estimate and 95% credible intervals of the association between oligotypes and susceptibility estimated in a single model that included those oligotypes with 90% credible intervals did not cover 1 in panel *A*. Abbreviation: PCR, polymerase chain reaction.

Associations With Age, Household Size, and Vaccination Status

We found that child household contacts (<18 years) were more susceptible to influenza A(H3N2) and B infection than adult contacts (≥18 years), with a risk ratio of 3.1 (95% CrI, 1.7–6.1) and 9.1 (95% CrI, 3.9–23.4), respectively (Figure 3, Supplementary Table 4). We estimated that influenza vaccine protected household contacts against influenza B virus infection, with a risk ratio of 85% (95% CrI, 11–99%), but not for influenza A(H3N2) virus infection (Figure 3, Supplementary Table 4). Residing in a household with 5 or more people was associated with a 48% (95% CrI, −1% to 73%) and 73% (95% CrI, 40–88%) lower risk of influenza A(H3N2) and B virus infection, respectively, compared with those in smaller households (Figure 3, Supplementary Table 4).

Serial Interval

We estimated that the mean serial interval for within-household transmission for influenza A(H3N2) and B virus infection was 2.4 days (95% CrI, 1.1–3.9 days) and 3.1 days (95% CrI, 0.9–6.6 days), respectively (Figure 4).

DISCUSSION

Compared with the earlier analysis using a mixed-effects regression model [22], the current transmission analyses advance our understanding about the association between the nasal/throat microbiome and the risk of influenza virus infection by providing influenza-type–specific associations in a household transmission setting. We identified bacterial community types and oligotypes associated with the susceptibility

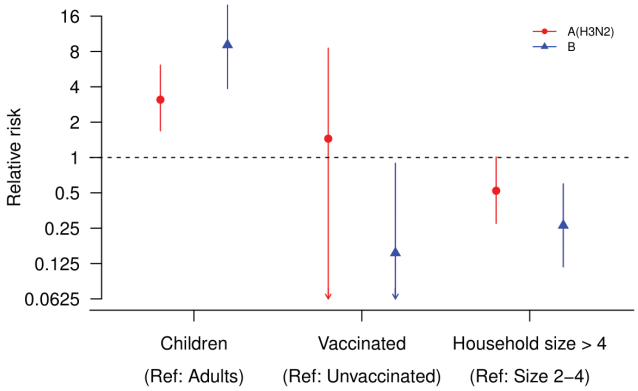

Figure 3. Effect of age group, vaccination status, and household size on transmission estimated under the household transmission model. Circles (red) and triangles (blue) were for household contacts with index cases with PCR-confirmed influenza A(H3N2) and B virus infection, respectively. Abbreviations: PCR, polymerase chain reaction; Ref, reference.

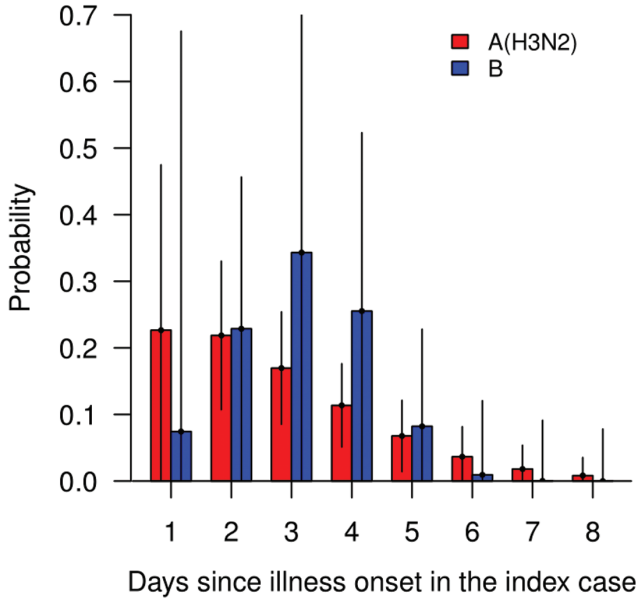

Figure 4. Serial interval distribution estimated under the transmission model, by influenza type, accounting for tertiary infections and infections from outside households. The estimated mean serial intervals for influenza A(H3N2) and B virus infection were 2.4 days (95% CrI, 1.1–3.9 days) and 3.1 days (95% CrI, 0.9–6.6 days), respectively. Abbreviation: CrI, credible interval.

to influenza A(H3N2) and B, while adjusting for age, household size and vaccination status. Our framework allows for imputing missing community types to include more households in the analyses to increase the power for detecting associations.

According to the transmission analyses, the community type but also the oligotype of the nasal/throat microbiome were associated with susceptibility to influenza A(H3N2) and B. These associations differ between influenza subtypes. Our findings are consistent with mouse models demonstrating associations between mouse commensal microbiota within the respiratory tract and susceptibility to influenza infection [5, 6].

The abundance of *Streptococcus* spp. was associated with lower susceptibility for influenza A(H3N2) and B infection in our analyses. This is consistent with previous studies showing that stimulation of the immune system by *Streptococcus* colonization may inhibit influenza viral replication [24–26]. In a mouse model, prior *S. pneumoniae* infection protected against severe influenza virus infection [26]. Among healthy young adults inoculated with live attenuated influenza vaccine, *Streptococcus infantis* was positively associated with influenza H1 immunoglobulin A (IgA) titers [27].

In contrast, the abundance of *Prevotella* spp. was associated with increased susceptibility to influenza B but not to influenza A(H3N2). *Prevotella* has previously been associated with increased severity of influenza, tuberculosis, and chronic obstructive pulmonary disease [28–30], but this is the first time its association with susceptibility to influenza has been detected. More in vitro or in vivo studies are needed to verify whether and how *Prevotella* modify host innate immunity or immune response specific to influenza viruses.

Our dataset has been analyzed without microbiome data [15] based on models without the subtype differences. Here, the microbiome data were added to the model and the estimates for factors affecting transmission were similar, suggesting that the association between microbiome and susceptibility may be independent of those factors. Consistent with the literature [31–33] and our previous analysis [15], we estimated that the mean serial interval for influenza B virus is 3.1 days, longer than the 2.4 days estimated for influenza A(H3N2) virus. Also consistent with the literature and our previous analysis [15], children were more susceptible to influenza A(H3N2) and B virus infection than adults [12, 21, 31, 34] and the differences for influenza B were more extreme than H3N2 [31, 35]. Potential explanations are the lower levels of pre-existing immunity among children, their higher frequency of contact and hence higher risk of exposure to influenza [36], or an inherent difference in the transmissibility between influenza A and B viruses [37]. Also, we found that smaller households were associated with higher secondary attack rate, which was consistent with our previous report [15] and other studies [20, 34].

In our study population, vaccine effectiveness for influenza A(H3N2) was much lower than for influenza B, which is consistent with a meta-analysis published in 2016 [3]. Other studies suggest that this difference may be due to poor vaccine-induced protection in some hosts [38] or mismatch of vaccine strain and circulating A(H3N2) strains due to egg adaptation [39].

Our study has several limitations. Index cases were recruited from health center attendees whose influenza was detected using a rapid test, suggesting they had more severe symptoms and higher transmissibility than general influenza cases. The enrollment of index cases was limited to 2 days or fewer after

symptom onset. As adults with illness usually tend to present later to clinics than children, our study likely has an overrepresentation of child index cases. Some tertiary infections may have been missed since the duration of follow-up was from 9–12 days after recruitment. However, the effect of this right-censoring should be minimal since the mean serial interval was around 3 days [15, 21]. Diet information was not collected, and hence the effect of diet on the nose/throat microbiome could not be explored. Infections were defined by PCR positivity, with no culture results. While PCR is the gold standard, it may detect infections with low infectiousness [40]. Finally, although important risk modifiers were adjusted for in our analyses [20, 34], we cannot be sure that detected associations are causal as there could be other important unmeasured confounders. A major strength of our study was that up to 5 respiratory samples were collected from household contacts over a period of 9–12 days after enrollment, regardless of symptoms. Therefore, the likelihood of missing infections due to peak viral shedding occurring between collection of sequential respiratory samples under such an intense sampling should be small.

In conclusion, we found that some bacterial community types and oligotypes of the nose/throat microbiome were associated with susceptibility to influenza. Importantly, these associations were dependent on influenza virus type/subtype. Our results suggest that the microbiome may serve as a useful predictor for susceptibility and have an implication for an alternative approach to the prevention of influenza infection via modulating the microbiome in the upper respiratory tract, should these associations be causal.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors thank the families that participated in the study and our excellent study staff both at the Health Center Sócrates Flores Vives and the Centro Nacional de Diagnóstico y Referencia.

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, under grant numbers U01AI088654 and R01AI120997 (to A. G.), grant number R21AI119463 (to B. F. and A.G.), and contract number HHSN272201400006C (subcontract to A. G.) and the Center for Statistics and Quantitative Infectious Diseases (grant number U54 GM111274-01) (to T. K. T.).

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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