

# Genetic Data Provide Evidence for Wind-Mediated Transmission of Highly Pathogenic Avian Influenza

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**Outbreaks of highly pathogenic avian influenza in poultry can cause severe economic damage and represent a public health threat. Development of efficient containment measures requires an understanding of how these influenza viruses are transmitted between farms. However, the actual mechanisms of interfarm transmission are largely unknown. Dispersal of infectious material by wind has been suggested, but never demonstrated, as a possible cause of transmission between farms. Here we provide statistical evidence that the direction of spread of avian influenza A(H7N7) is correlated with the direction of wind at date of infection. Using detailed genetic and epidemiological data, we found the direction of spread by reconstructing the transmission tree for a large outbreak in the Netherlands in 2003. We conservatively estimate the contribution of a possible wind-mediated mechanism to the total amount of spread during this outbreak to be around 18%.**

**Keywords.** avian influenza; molecular epidemiology; mathematical modeling.

Avian influenza is endemic in many wild bird species, which harbor all known subtypes of influenza A viruses. The virus can be transmitted from wild birds to poultry, thereby crossing the species boundary. Although most virus strains cause no or few clinical symptoms in poultry, highly pathogenic (HPAI) variants can arise through mutation [1–3]. These highly virulent strains, the most notorious of which is HPAI H5N1, can cause large outbreaks with high mortality, posing a major (economic) threat to poultry farming around the globe. In addition, the virus can cross over

to human hosts, potentially resulting in severe disease or even death [4]. Therefore, HPAI is considered a serious public health threat [5, 6].

There have been several large outbreaks of avian influenza in Western countries, involving clusters of large commercial poultry farms. Due to the ease with which the disease seems to spread between farms, the high mortality rates among poultry, and the public health threat posed by an outbreak [7], rigorous control measures must be implemented. These typically consist of a complete transport ban of poultry and depopulation of all farms that either have infected animals or are at risk of infection, resulting in substantial economic losses.

Poultry farms emit large quantities of particulate matter [8, 9], which could be driven by wind to transport viable virus from an infected farm to an uninfected farm [10]. However, opinions differ widely on whether this actually causes new infections during an outbreak; the mechanism has never been demonstrated conclusively [10–13]. Humans, trucks, or wild birds could also act as a vector, carrying the virus between farms [14].

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Knowledge of the actual transmission mechanisms and their relative importance could lead to more efficient and more effective control strategies. For example, enforcement of stricter biosecurity procedures could reduce spread by humans, and adjustment of ventilation systems could combat wind-mediated spread. Insight into the mechanism of spread would also lead to more precise estimates of which farms are at risk of infection. This knowledge is highly valuable during an outbreak, for example, when planning the order in which to cull farms [15].

Here, we use detailed genetic and epidemiological data from an outbreak of HPAI A(H7N7) in the Netherlands in 2003 to test the hypothesis that wind aided in transmission of the pathogen. In this outbreak, 241 poultry farms were infected (confirmed by virus isolation), 30 million birds were culled, and there was 1 human fatality [16, 17]. Isolated virus RNA was sequenced for 231 of the infected farms [7, 18]. These unique genetic data, in combination with time of infection and time of culling, allowed us to determine which farms infected other farms. To test the role of wind, we compared the direction of these individual farm-to-farm transmission events with the wind direction at the date of infection, accounting for any bias induced by the geography of the farm locations. We conclude by giving an estimate for the percentage of infections that can be attributed to wind-mediated transmission.

## METHODS

There were 5360 poultry farms in the Netherlands in 2003; geographical information is available for all of these farms. Flocks were culled for 1531 farms, and the date of culling is known for all farms. For 227 of the 241 infected farms the date of infection has been estimated, based on mortality data [19]. The remaining 14 farms are hobby farms, defined as farms with fewer than 300 animals, for which no mortality data are available. For these farms, we use the infection date as estimated by Boender et al [20]. The HA, NA, and PB2 genes of viral samples from 231 farms have previously been sequenced [7, 18]. Sequence data can be found in the GISAID database under accession numbers EPI\_ISL\_68268–68352, EPI\_ISL\_82373–82472, and EPI\_ISL\_83984–84031. Available meteorological data include wind speed and direction (with a 10-degree precision) for every hour of every day of the outbreak, measured at 5 weather stations close to the infected farms (Figure 1). These data are available from the Royal Dutch Meteorological Institute at [www.knmi.nl](http://www.knmi.nl).

### Estimation of Transmission Events

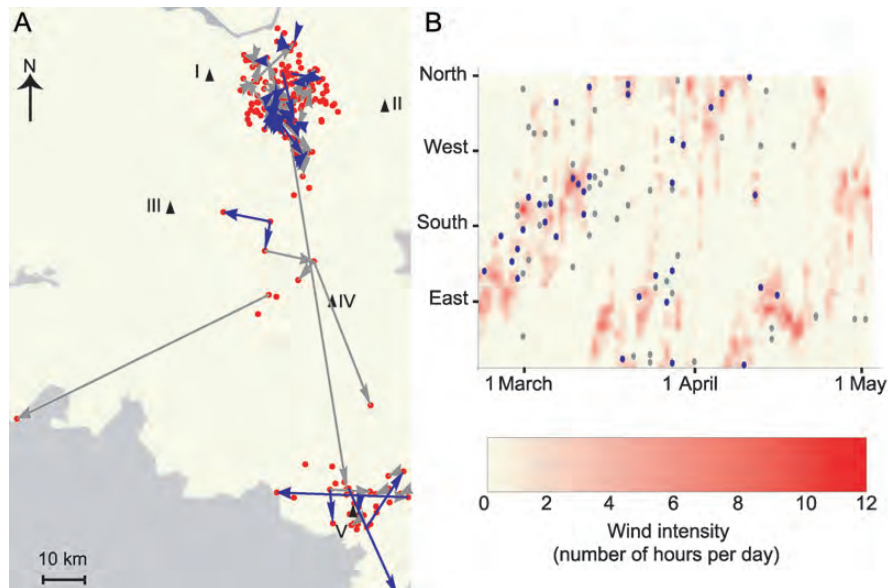
To determine which farms infected other farms, we used the genetic and temporal data on the infected farms, following the method described by Ypma et al [21]. We described the likelihood of a possible transmission tree, given the data, by

arguing that the tree is more likely if the source farms are more infectious at the putative dates of infection and if the total number of mutations needed to explain the genetic data is lower. This is done using a simple substitution model that differentiates between transitions and transversions [18, 21]. We then sampled from the space of all transmission trees using a Markov chain Monte Carlo approach and obtained the probability of a certain transmission event by the proportion of sampled trees that includes this event. We denote transmissions with a posterior probability of at least 0.9 as observed transmissions; results for different cutoff values are similar (Supplementary Information, section 4.1).

### Correlation of Wind and Transmission Directions

To measure whether observed transmissions were in the same direction as the wind, we compared the direction of transmissions with the wind direction. We took the vector average wind direction measured at the station closest to the infecting farm at the date of infection. To check for a correlation between the wind direction and direction of transmission, we calculated the circular correlation coefficient [22]. We then compared the value of this coefficient with values obtained under the null hypothesis that wind direction and direction of transmission are independent and uniformly distributed over all directions.

A correlation between wind and transmission could arise as an artifact of the geographical location of poultry farms. For instance, if the index farm of the outbreak lies west of an area dense with poultry farms and if there was a prevailing westerly wind in this region, we could obtain a correlation even in the absence of any causal relationship. We used simulations to construct the correct null model for the relation between direction of transmissions and direction of wind when wind plays no role. We used the coordinates of poultry farms corresponding to the Netherlands in 2003 and infected the farm corresponding to the index farm in the real outbreak. Every farm infected during the first 10 days of the simulation was culled (and thus removed from the simulation) 10 days after transmission, as were all farms in a 1-km region around the infected farm 2 days later. Farms infected later than 10 days into the simulation were culled after 7 days, ring culling again following 2 days later. The probability of infecting another farm decreased with distance, as estimated previously [20, 21]. Only simulations that led to a total number of infections between of 200 and 280 were used for the subsequent analysis. From each simulated outbreak we randomly sampled as many transmission links as there were observed transmissions in the actual data. We used the sampled transmissions from 1000 simulations to calculate correlation with wind direction. In addition, we compared the simulation results with the actual data using 2 additional statistics that are relevant for a possible wind-mediated mechanism of spread. First, we looked at the



**Figure 1.** Observed transmission events of avian influenza A(H7N7) between farms in the Netherlands, 2003. Transmissions that coincide with wind direction (measured at the closest meteorological station) are in blue. Observed transmissions are defined as transmissions estimated at a posterior probability of at least 0.9. (A) Transmission events on a map of the region. Dots denote infected poultry farms; triangles denote the 5 closest meteorological measuring stations. (B) Density plot of wind direction (measured at station IV) against time, with red regions indicating wind is predominantly in 1 direction. Dots denote estimated time and direction of transmissions. Wind direction and direction of transmissions appear to be correlated. For example, at the start of the outbreak, around 1 March, wind direction was predominantly southeast (red region); during this time period, most transmission events were also in this direction (dots).

angle between wind and transmission by taking the cumulative distribution function of  $P(x)$ , the probability that the angle between transmission and wind direction is  $x$ , and performed a one-sided Kolmogorov-Smirnov test to see if this distribution was significantly larger for the actual dataset. Next, we compared the average number of hours that wind coincided with the direction of a transmission, which could be interpreted as the time window for transmission due to wind.

### Quantification of Wind Contribution to Spread

We quantified what proportion of the transmission events could be attributed to a wind-related mechanism of transmission, assuming that such a mechanism existed. Here we use the term “wind-mediated transmission” to denote transmission events that can be attributed to a wind-related mechanism of transmission if such a mechanism would exist.

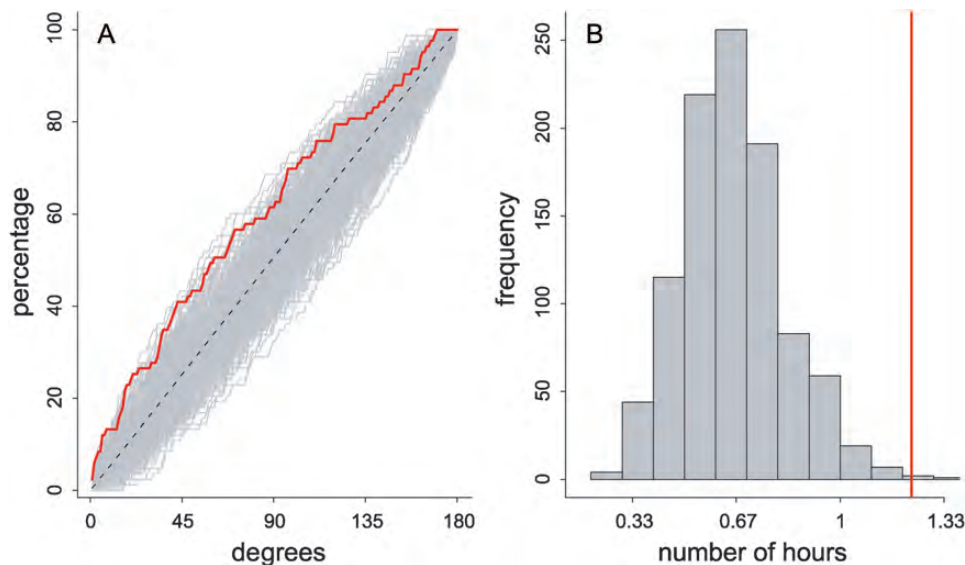
An observed transmission was defined to be in the direction of the wind when the average wind direction was in the direction of spread, up to 5 degrees, for at least 1 hour on the date of infection. The proportion of transmissions mediated by wind could then be estimated by comparing the fraction of observed transmission events that are in the direction of wind with the fraction of transmissions expected to be in the direction of the wind if wind played no role. This expected fraction can be found from our simulations. The observed

fraction is a combination of transmissions actually due to wind and some that were in the same direction as wind due to chance. From this we estimated the percentage of transmissions mediated by wind (see Supplementary Information, section 3.1, for details).

To test the robustness of this simple estimation procedure, we performed a more detailed analysis that used all available data but needed additional assumptions. In this approach, uncertainty in estimated infection dates was accounted for by using a prior of several days centered on the estimated date. The probability per day of infecting a farm at a certain distance can be found by looking at the ratio of the number of farms at that distance that were infected and that could have been infected [20]. Here, we took this distance-related probability to consist of a “wind-related part”  $W$ , assuming a Gaussian plume model for wind-related spread [23], and an “unknown mechanism part”  $U$ . The percentage of transmissions related to wind can then be estimated as the  $W/(U + W)$  (see Supplementary Information, section 3.2, for details).

## RESULTS

For all farms infected during the avian influenza A(H7N7) outbreak, we identified the most probable infecting farm using



**Figure 2.** Comparison of observed transmissions with simulations in which transmission was unrelated to wind. (A) The cumulative percentage of transmissions having an angle with the vector average wind direction of a certain degree, for observed transmission events (red) and 1000 simulations (grey). The distribution for the dataset lies significantly outside the range generated by the null hypothesis given by the simulations (1-sided Kolmogorov-Smirnov test,  $P < .01$ ). (B) The average number of hours for which wind direction was equal to direction of spread at the date of infection. Because there are 24 hours in a day and hourly average wind directions are available with 10-degree precision, we expect the mean number of hours when wind plays no role to be  $24/(360/10) = 2/3$ . The histogram gives the values for the simulations; the red line gives the value for the observed transmission events. Both graphs show that the correlation between wind direction and direction of spread cannot be explained by chance or farm geography alone.

infection date, culling date, and viral RNA sequence data of the HA, NA, and PB2 genes. We identified a single infector farm with a probability of at least 0.9 for 83 farms; we call these 83 pairs of infected and infector farms the “observed transmissions.” Figure 1A shows the location of the farms, the distance over which the observed transmissions occurred, and the direction of observed transmissions on a map of the region. Most transmissions occurred over short distances in a central high-density farm area. Figure 1B shows the wind direction over the course of the outbreak together with the direction of the observed transmissions.

The circular correlation coefficient between direction of observed transmissions and wind direction was 0.051, significantly higher than expected when directions were uniform and independent ( $P = .01$ ) (Supplementary Figure 1). The circular correlation coefficient was also significantly higher than expected based on geography of farm locations; we found the 0.975 quantile for the circular correlation coefficient under the simulations to be 0.043. We further found that the angles between the direction of observed transmissions and the vector average wind direction at the date of infection were significantly smaller than the angles between the direction of simulated transmissions and wind direction (1-sided Kolmogorov-Smirnov test,  $P < .01$ ) (Figure 2). Likewise, the average number of hours for which wind was in the same direction as the observed transmission at the date of

infection was significantly higher for the actual dataset than for the simulations. We therefore conclude that the correlation between direction of transmission and direction of wind is higher than can be explained by chance and location of farms.

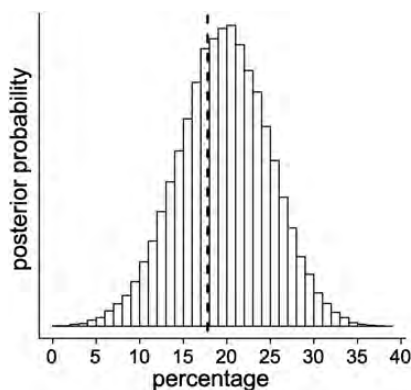
The strong positive correlation between wind direction and direction of influenza transmission suggests that a substantial proportion of the transmission events are mediated by wind. To estimate this proportion of transmissions mediated by wind, we compared the percentage of observed transmissions in the direction of the wind with the percentage expected by chance. In our analysis of the actual data, 34% of the posterior probability was on transmissions in the direction of the wind. We assumed this 34% to be made up of transmissions that were and transmission that were not mediated by wind. The first would all be observed to be in the direction of the wind, while of the latter, only a percentage would be observed to be in the direction of the wind by chance. From our simulations we know that, on average, 24% of transmissions not related to wind will be in the direction of the wind by chance. Using these numbers and maximizing a likelihood equation (see Supplementary Information, section 2), we estimated the percentage of transmissions caused by wind to be 18% (95% confidence interval (CI), 6.3, 30). To test for robustness, we also performed a more detailed analysis, which assumes a Gaussian plume model for wind spread, puts a priority on the infection

dates, and takes uninfected farms into account. From this model we obtained an estimate of the percentage of transmissions of 20% (95% CI, 9.8, 29), which is consistent with the first analysis (Figure 3).

## DISCUSSION

We have shown that for the outbreak of HPAI A(H7N7) in the Netherlands in 2003, inter-farm transmissions are more often in the direction of the wind than can be explained by chance and coordinates of poultry farms. Based on the proportion of estimated transmissions for which wind direction was observed to be in the same direction of spread, we estimated the percentage of transmission related to wind to be 18%.

Wind-related spread of avian influenza has direct consequences for containment efforts. Farms emit vast quantities of particulate matter [8], which could carry viable virus [13]. Several techniques, such as air scrubbers, water or oil sprinkling, changes in ventilation rate, and ionization systems, have been shown to reduce dust concentrations [8, 24] and could be an efficient way to stop infectious particles from getting in or out of a farm. Alternative wind-related mechanisms cannot be excluded on the basis of our analysis. Wild birds or insects acting as vectors for the disease [25], flying preferentially in the direction of the wind [26, 27], would explain our observations as well, but call for different control strategies. Furthermore, culling strategies may take into account the role of wind. First, care should be taken to ensure contaminated material does not get into the environment during culling activity. Second, wind direction should be taken into account when



**Figure 3.** Estimation of the percentage of transmissions mediated by wind. A comparison of the fraction of observed transmissions in the direction of wind with the fraction expected when wind plays no role yields an estimate of 18% (dashed line). Using a mechanistic model, which assumes a Gaussian plume model for wind-related spread, yields an estimate of 20%. The full posterior distribution is given by the bars.

estimating the risk of infection for farms; the most efficient culling order will first target those farms that are at higher risk of infection and, when infected, will pose higher risks to other farms [15]. Thus, increased knowledge of which farms are at risk, provided by current and forecasted wind direction, allows for a more efficient culling strategy.

There are several sources of error in our estimation of the transmission tree. The assumed constant infectiousness of farms over the course of infection, the substitution model, and the assumption of independence between mutations and time are simplifications. Furthermore, there is uncertainty in the estimation of the infection dates, and there may be errors in the geographical and genetic data. These limitations will lead to errors in the inference of transmission events. Counterintuitively, these limitations only strengthen our conclusion. If wind-mediated transmission played only a minor role, there is a negligible probability that the cumulated small errors in the data and in the inferential procedures could have produced the observed strong positive correlation between wind direction and direction of transmission. The main reason for this is that the transmission tree was reconstructed without using the meteorological data; only afterward were transmissions compared to wind directions. It is much more likely that the cumulated errors would reduce any existing strong positive correlation. Therefore, our conclusion of wind-mediated spread holds in the presence of small errors, and the value of 18% should serve as a lower bound for the actual percentage of transmissions related to wind.

The type of analysis presented here may have potential to identify and quantify transmission mechanisms for other farm animal diseases. However, the resolution we obtained here, tracking individual transmissions, could only be achieved through the high percentage of farms sampled and the high genetic diversity found. This resolution was necessary; an analysis looking solely at prevailing wind direction and farm coordinates would not have shown any significant effects. We therefore believe that efforts should be made to gather genetic data for outbreaks of other infectious diseases as well. We do, however, note that usefulness of such data will depend on the genetic diversity that accumulates over the course of the outbreak, which might be lower for other pathogens and depend on the methods used.

Identifying the mechanisms responsible for the transmission of livestock disease between farms is challenging for the following reasons: first, because data have to be collected during the outbreak, when the first priority is control rather than research, and second, because there are probably several different mechanisms at play, making their identification troublesome. The key to identifying transmission mechanisms is the reconstruction of detailed transmission networks, made possible by the joint analysis of detailed genetic and epidemiological data.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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**Potential Conflicts of Interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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