Mechanisms of Avian Influenza virus transmission between farms: combining data collection and mathematical modelling

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Thesis

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Mechanisms of Avian Influenza virus transmission between farms: combining data collection and mathematical modelling

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For the inspiration of Bitiyali, Shivan, Sheenah and Ethan

Propositions

1. A farm is still infectious even when it no longer exists (This thesis: Chapters 5 and 6).

2. Farms visited by the people in white coverall (call them crisis organisation teams) are the most likely to be infected but these people are the least likely to have caused this infection (This thesis: Chapter 3).

3. Without models (mathematical or statistical), data is nothing more than just some numbers or noise (adapted from Chris Anderson, wired magazine 2008).

4. In managing disease epidemics, it is equally important to know more about the transmission dynamics of a pathogen as it is to have an efficacious drug against the pathogen.

5. The frequency of social gatherings (BBQs and drinks) is directly proportional to the quality and progress of scientific research.

6. Good quality research is related to the mode of travel to meetings: asking a senior scientist for a ride is the best option to obtain new scientific ideas.

Propositions belonging to the thesis: “Mechanisms of Avian Influenza virus transmission between farms: combining data collection and mathematical modelling”

Amos Ssematimba
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Abstract

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The lack of sufficient knowledge on the mechanisms of between-farm spread of livestock diseases hampers the development of much needed effective and fast control strategies. Some of the mechanisms responsible for pathogen spread can be deduced from epidemic tracing reports and literature while others can only be hypothesized from findings of studies on daily farm practices throughout the production round. For outbreaks without known/traced transmission routes, the concept of 'neighbourhood' infection is often adopted. This concept was founded based on the distance-dependence of the transmission risk with geographical proximity to an infectious farm being the key determinant of risk. Mathematical modelling plays an important role in obtaining quantitative insights into the contributions of the different mechanisms to disease spread. This can be by ranking the contributions of the individual transmission routes and/or obtaining a generic distance-dependent transmission risk. The models can guide the design of control strategies by providing a means to assess the efficacy of intervention strategies. In this thesis, modelling was used to assess the contributions of the wind-borne route and the other (traced) between-farm contacts to the transmission of highly pathogenic avian influenza during an epidemic in the Netherlands in 2003. It was found that these two routes together could only explain approximately 31% of the infections/cases. Visits by epidemic control teams were the least risky indicating the effectiveness of their biosecurity protocols in preventing transmission. New data on day-to-day farm practices and farmer opinion was collected in an attempt to generate hypotheses on transmission pathways and mechanisms that were yet to be appreciated. Indeed relevant unappreciated practices were found. They include irregularities in compliance to biosecurity as well as a broad category of neighbourhood-related risks. A new modelling approach to study neighbourhood transmission was developed guided by indirect transmission experiments. It involves the approximation of the pathogen dispersal process by a diffusive transport mechanism. Applying this diffusion model to the outbreak data of 2003, it was found that assuming delayed transmission, as opposed to instantaneous transmission, is an important phenomenon to be considered when modelling disease spread between locations. This modelling approach has the added advantage of availing an opportunity to assess the performance of intervention strategies without detailed mechanism-specific information.

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Chapter 1

General introduction

Highly Pathogenic Avian Influenza

Background on outbreaks in poultry and humans

Highly Pathogenic Avian Influenza (HPAI) is among the World Organisation for Animal Health (OIE) listed diseases. Its first description dates back to 1878 in Italy [1].The first report of an HPAI outbreak caused by a virus of H5 subtype was in 1959 [2] and more have been reported since then. H5 and H7 are so far the only subtypes that are highly pathogenic in poultry. HPAI viruses in poultry evolve from Low Pathogenic Avian Influenza (LPAI) viruses that are common in wild waterfowl [3-5]. The virus may enter into poultry as a LPAI strain and subsequently evolve into an HPAI strain. The 1999 H7N1 epidemic in Italy [6] and the 2003 H7N7 epidemic in the Netherlands [7,8] are examples of the devastating HPAI epidemics involving H7 subtype strains that are said to have evolved from LPAI strains. Alexander and Brown[9] mentioned that HPAI virus emerged independently at least 11 times of which four epidemics involved millions of birds. Consequences of these epidemics are enormous and include among others: a high risk of spread to other farms [10], high mortality rates, economic losses incurred in implementing control strategies and reduction in exports [11,12] and, above all, a risk of spread to humans for some strains (both of the H5 and H7 subtypes) [7,13].

One of the most catastrophic influenza pandemics in the previous century is the 1918-1919 Spanish flu which resulted into between 20 and 50 million human fatalities[14]. Because of this catastrophic epidemic, the panzootic and zoonotic characteristics of the H5 and H7 subtype strains have raised public health concern [3]. It is hypothesized that the H5 and H7 subtype strains may evolve into future pandemic human strains [5,15,16]. The first outbreak of an avian influenza A virus strain (of the H5N1 type) in humans occurred in 1997 Hong Kong, affecting at least 18 individuals, six of whom died [17]. From 2003 until 2nd May, 2012, a total of 603 human cases of influenza A/H5N1 type strains, different from the 1997 Hong Kong strain, have been reported with 356 deaths registered. For the H7 subtype strain, during the Dutch 2003 epidemic, 89 people were infected one of whom died [7]. Therefore, thorough knowledge on the dynamics of avian influenza viruses and on the control of its epidemics is not only important to livestock industry but also for public health.

The Dutch 2003 H7N7 HPAI epidemic

In 2003, an H7N7 HPAI epidemic occurred in the Netherlands and spread to a few farms in Belgium and Germany. In the Netherlands, the virus was isolated from 241 flocks and 14 flocks were serologically positive and approximately 30 million birds were killed [7,8,18]. Specifically 168 layers, 18 turkeys, 34 breeders, two broilers, two ducks, 13 pets and four categorized as others were affected [19]. The virus affected eight farms in Belgium and one farm in Germany [20,21]. The OIE and European Commission guidelines and regulations to control HPAI epidemics include among others a ban on transport of live poultry and poultry products, and the implementation of strict biosecurity measures. In reference to the Dutch 2003 epidemic, following diagnosis of the first cases in late February, movement bans were implemented after five days and other control measures followed. However, more farms became infected and in the second week of March the preventive culling of contiguous flocks was introduced[8]. The direct costs such as costs of lost birds and costs of controlling the epidemic in the

Netherlands alone were more than 250 million euros, whereas the indirect costs such as lost markets were even higher [11,12].

This epidemic was characterized by high attack rates, high mortality and a rapid spread to naïve farms through untraced transmission routes [8,10,22]. More than 80% of the cases of the Dutch epidemic are reported to have occurred through spread between farms by untraced routes [8,10,22,23]. Pathogens can be spread through both 'direct' and 'indirect' contacts. Direct contact involves the introduction of infected animals onto a susceptible farm whereas indirect contacts involve the transfer of infectious material between farms by other routes than live animals. Transmission during most of the epidemic is likely to be dominated by indirect routes as this transmission occurred in the presence of a ban on animal movement. This indirect transmission may probably be associated with human or fomite involvement by transferring infective organic material such as manure between locations [2,5,22,24-26]. The continued spread of the HPAI during epidemics may indicate the existence of unappreciated mechanisms. It is likely to be aided by transmission routes/mechanisms (untraced) that are neither controlled by the control strategies nor by the enhanced biosecurity. This spread is also referred to as 'neighbourhood' transmission. For the Dutch epidemic, the transmission risk was found to increase with decreasing distance to infected farms (Figure 1) and therefore the risk was higher for farms in the high poultry farm density areas [10].

Stegeman et al. [8] concluded that the epidemic was only contained due the reduction in the number of susceptible flocks by complete depopulation of the affected areas and not likely to have been due to a reduction in transmission by other control measures. However, the massive preventive killing of animals is criticized more and more, mainly on ethical grounds. One of the reasons for the use of massive slaughter as a means to prevent further transmission of the pathogen may be the lack of substantive knowledge about the underlying mechanism(s) of neighbourhood transmission of the infection. Yet the development of alternative control strategies, in particular individual biosecurity measures, requires more insight into these underlying mechanisms [22]. This is because, in contrast to measures that eliminate susceptible farms which have the same effect on all transmission routes, improving biosecurity typically acts on a subset of the between-farm transmission routes. More insights into the between-farm disease mechanisms can be gained through a combination of experimental and modelling work [27]. For example, mathematical models can be used to extrapolate findings from experimental studies on indirect transmission to infer about the general problem of neighbourhood transmissions between farms.

Mathematical models as a tool to guide the design of control strategies

Mathematical models play an important role in understanding the dynamics of infectious diseases [27-30]. They can be used to guide predictive and contingency planning during epidemics [31,32]. They provide a means to interpret data from past and on-going epidemics as well as experiments by aiding the estimation of disease- and epidemic-related parameters. The quantitative information obtained helps to improve preparedness for future epidemics. This information can be used to estimate the required vaccination coverage to achieve herd immunity as well as to guide the assessment of the efficiency of newly proposed control strategies. For example the role of interventions such as improved biosecurity can be assessed through quantifying transmission probabilities and incidence rates.

Using data from the 2003 HPAI epidemic in the Netherlands, models have been used to estimate important parameters such as the transmission rate, the basic reproduction ratio, the date of introduction of the virus on to the farm and to assess the effectiveness of control strategies [8,10,33,34]. Other examples on the use of models and data from other epidemics can be found in [35-39]. Quantitative information provides a basis for the design of control strategies for HPAI epidemics. Through a review on avian influenza modelling literature, de Jong and Hagenaars [40] describe how quantitative information can be used to guide model building, parameter estimation and model validation. They emphasize the importance of combining modelling with data (from outbreaks and/or experiments) in order to generate this information.

Mechanistic and statistical modelling

Through quantitative approaches such as modelling, mechanistic insights into the spread of infectious diseases are gained. This insight is needed to improve intervention strategies during epidemics. Mathematical models are generally grouped into analytical and simulation models with the former being further subdivided into the mechanistic (stochastic or deterministic) and statistical models [40] although some statistical techniques such as the Bayesian approaches may involve simulation. Therefore, typically, analytical models are not necessarily fully analytically solvable and are often times evaluated in part analytically (e.g. in the calculation of R_0) and in part numerically. Numerical evaluation can be exact (almost always possible for deterministic models) or through model simulation (often necessary for stochastic models).Statistical and spatiotemporal modelling and analysis techniques are used to generate risk maps for geographical disease spread as well as to determine the critical farm density for spread among others [10,28,41-43].

In relation to HPAI spread, this approach has been used by Boender et al. [10] to analyse the 2003 Dutch epidemic and also by Dorigatti et al. [41] to analyse the 1999 H7N1 Italian epidemic. The transmission kernel (depicting the distance-dependent transmission risk) obtained in the Boender et al.[10] study is shown in Figure 1. That study aimed at determining the distance-dependence of the overall transmission risk as well as to generate risk maps for HPAI spread. No attempt was made to assess the contribution of the individual mechanisms to this risk, an aspect that would largely benefit the development of better control strategies. For example, if derived, the route-specific quantitative information can be used to parameterise mathematical models that assess the efficacy of intervention strategies such as improved biosecurity against those mechanisms during epidemics.

Using mechanistic approaches as opposed to the statistical ones provide an opportunity to gain deeper understanding into the mechanisms underlying neighbourhood transmission. In this thesis, an attempt to ‘break down’ the transmission kernel (Figure 1) into its constituent mechanisms based on the outbreak and modelled data is made.

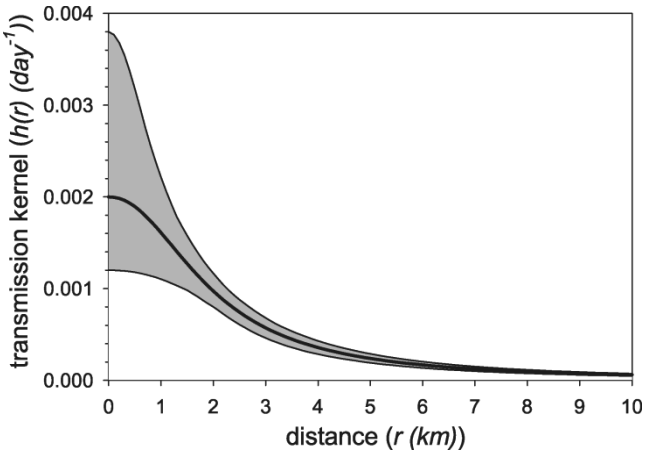


Figure 1. The transmission kernel as a function of inter-farm distance (Obtained from Boender et al. (2007))

Mathematical modelling and other approaches in this thesis

In this thesis, a comprehensive approach is adopted to analyse the Dutch 2003 epidemic data to quantify the possible contributions of various between farm links to the overall transmission risk (Figure 1). The dispersal mechanisms may include the traced and untraced between farm contacts as well as the other untraceable (i.e., mechanisms that are impossible to trace for example rodents and insects that may move between farms) and indirect mechanisms such as the wind-borne route. The contribution of the direct contacts (human and fomites) –hence the assumption of instantaneous transmission– and the windborne route is determined. Thereafter an attempt is made to fill the gaps in the known (and hence traced) contacts by collecting more data. Lastly, the possibility of between farm spread being a combination of instantaneous and delayed transmission–in which pathogen dispersal is approximated by a step-by-step diffusion process is investigated.

The quantitative contributions to disease spread of the between-farm contacts during the Dutch 2003 HPAI epidemic are determined. They are quantified through per-contact probabilities of virus transmission as well as through estimating distance-dependent transmission probabilities. In addition to that, the proportion of cases explained by each of these routes is determined. Figure 1 is used as a reference for the comparison to access the proportion explained by a given route for example the windborne route. New data on possible HPAI transmission routes and mechanisms was collected to guide the elucidation of new pathways and mathematical and statistical models are used to analyse this and the existing data from the epidemic.

When analysing epidemic data, it is difficult to make the link between the timing of individual between farm contacts and the onset of infection at the receiving farms. The lack of knowledge on the exact timing of onset of infection as well as the possibility of long distance transmission motivate the search for more insight on the dispersal mechanisms of pathogens between farms. For example, for long distances, transmission may not be instantaneous because infectious material may take time to disperse between the two farms. To explore this possibility, the original assumption of instantaneous transmission adopted in estimating the transmission kernel for the Dutch

2003 epidemic [10] is put to test. The effect of incorporating delayed transmission on the predictive power of transmission kernels estimated from epidemic data is also investigated.

Thesis aim and outline

Indirect transmission plays a role in the spread of livestock diseases between farms. The approaches developed in this thesis help in gaining more insight into the transmission mechanisms by already implicated and the newly hypothesized mechanisms. The main aim of this thesis was to gain more quantitative insight into plausible mechanisms underlying neighbourhood transmission of HPAI. The techniques developed throughout this thesis are also applicable in the study of indirect transmission of other livestock and human diseases. It is hoped that the outcomes of this study will guide the update and extension of the existing control measures against the spread of infectious livestock disease between farms.

In Chapter 2, mathematical models are used to assess the role of the windborne route in the between-farm spread of the virus. In Chapter 3, the contributions of the traced and modelled/unknown between-farm contacts during the 2003 epidemic are assessed through the quantification of their per-contact transmission probabilities and the estimation of the number of new infections they potentially caused. In Chapter 4, based on the findings from an interview study conducted in the Dutch poultry industry, new potential transmission pathways are hypothesized and a qualitative transmission risk assessment of these and the already known pathways is made. In Chapter 5, experimental and modelling approaches are combined to gain insight into indirect transmission. Pathogen dispersal is approximated by a diffusion process and a diffusion model is used to analyse transmission data from experiments. Finally in Chapter 6, the role of delayed transmission during epidemics is investigated. Spatiotemporal analysis on part of the Dutch 2003 HPAI outbreak data is performed by applying the diffusion model derived in Chapter 5 to the data to explore the use of the model as a description of the between-farm transmission of avian influenza during that epidemic.

Chapter 2

Modelling the Wind-borne Spread of Highly Pathogenic Avian Influenza virus between farms

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Abstract

A quantitative understanding of the spread of contaminated farm dust between locations is a prerequisite for obtaining much-needed insight into one of the possible mechanisms of disease spread between farms. Here, we develop a model to calculate the quantity of contaminated farm-dust particles deposited at various locations downwind of a source farm and apply the model to assess the possible contribution of the wind-borne route to the transmission of Highly Pathogenic Avian Influenza virus (HPAI) during the 2003 epidemic in the Netherlands. The model is obtained from a Gaussian Plume Model by incorporating the dust deposition process, pathogen decay, and a model for the infection process on exposed farms. Using poultry- and avian influenza-specific parameter values we calculate the distance-dependent probability of between-farm transmission by this route. A comparison between the transmission risk pattern predicted by the model and the pattern observed during the 2003 epidemic reveals that the wind-borne route alone is insufficient to explain the observations although it could contribute substantially to the spread over short distance ranges, for example, explaining 24 % of the transmission over distances up to 25 km.

Keywords: highly pathogenic avian influenza; windborne spread; Gaussian plume model; particle settling; pathogen decay; deposition

Introduction

Highly Pathogenic Avian Influenza virus (HPAI), Classical Swine Fever Virus (CSFV), and Foot-and-Mouth Disease Virus (FMDV) are highly contagious viruses affecting livestock and are among the World Organisation for Animal Health (OIE) listed diseases. The consequences of their recent epidemics in the Netherlands [8,44,45] have been enormous and include high mortality rates, economic losses incurred in implementing control strategies and reduced exports, and for HPAI, a risk of spread to humans [7,8]. During the 2003 HPAI epidemic in the Netherlands, following detection of the first outbreaks in late February, movement bans were implemented followed by other control measures. Nevertheless, more farms became infected and therefore in the second week of March the measure of preventively culling contiguous flocks was adopted. In the end, 255 flocks were affected over the course of the epidemic and close to 30 million birds were culled; in addition, the virus was transmitted to 89 people causing one fatality [7]. Between 80% and 90% of the outbreaks occurred through untraced routes, with the farm infection hazard increasing in the vicinity of earlier infected (but as yet undetected) farms [10,22]. The sustained between-farm transmission despite extensive control measures demonstrated the difficulty of controlling HPAI spread in poultry-dense areas.

The mechanisms underlying the between-farm spread of HPAI are not clearly understood, especially those of indirect transmission (involving vectors or fomites and possibly wind-borne transfer), as opposed to direct transmission (transportation of live animals between farms) [8,10,22]. Indirect transmission has played a major role in large epidemics involving viruses such as CSFV [46,47] and FMDV [42]. In the analysis of the Dutch 2003 HPAI epidemic data, Boender et al. [10] used statistical spatial-temporal modelling techniques and identified high risk areas for epidemic spread. The same technique of using a spatial transmission kernel was used by [42,48] in studies on the between-farm spread of FMDV in Great Britain. Although important insights, helpful for the development of control strategies laid out in contingency plans, were gained from these analyses, a lack of mechanistic (as opposed to statistical) understanding of the between-farm spread currently impedes the further improvement of these strategies. For example, the extent to which biosecurity measures on farms contribute to limiting indirect transmission is unclear, as is how these measures can be improved.

With stringent control measures put in place during epidemics including bans on the movement of animals, the direct spread of the virus is reduced. Therefore, indirect routes such as contamination of personnel and fomites do become the only pathway of virus spread. Indirect transmission could arise from human vectors transferring infective excreta such as manure from infected to recipient animals [24,25,49], mechanical transfer of excreta [5,22,24] or a possible combination of these mechanisms.

The need to determine whether wind-borne transportation of the virus is one of the untraced routes of HPAI spread between farms is apparent. The simplest way possible is that where the virus is transported by wind from an infected farm directly to an uninfected farm as has been considered in plume models for FMDV spread [50-54]. Otherwise, the dispersal may be through a multi-stage process. In such a process, the virus may be transported from infected animals to recipient animals by wind during certain parts of the route and by other means (for example humans and vehicles) on other parts. Both scenarios require quantitative insight into the deposition pattern of (contaminated) farm dust.

Davis et al. [55] conducted a study on the spread of Equine Influenza in Australia in 2007. They concluded that virus was spread over 1-2 km via wind-borne aerosols. However, the significance of wind-borne spread of HPAI is subject to divergent opinion. This lack of consensus was mentioned by Power [56], who also noted the absence of any testing to support or refute a wind-borne theory of HPAI spread during the epidemics in Italy and the Netherlands. This route is often considered insignificant, but with no serious underpinning based on quantitative evidence. For example, Swayne and Suarez [25] suggest that although aerosols and wind-borne contamination may have caused some secondary spread during the New South Wales HPAI H7N4 epidemic in 1997, they should not be regarded as important in the spread of infection. Yet in the analysis by Power [56] of the 2004 H7N3 AI epidemic in Abbotsford, BC Canada, air samples taken around the infected poultry houses confirmed the circulation of HPAI in the air outside the barns. This motivates our aim to quantitatively assess

whether, and to what extent, this route may have played a role in the Dutch 2003 HPAI epidemic.

We do this by developing a model for wind-borne transmission of HPAI between farms, and comparing its predictions for the distance-dependent wind-borne transmission risk with the observed transmission risk in the Dutch 2003 H7N7 epidemic [10]. In our analysis, where possible, we use the Dutch 2003 H7N7 HPAI strain to quantify HPAI-specific parameters such as the within-flock basic reproduction ratio R_0. In our model, we consider the wind-borne dispersal and deposition of farm dust contaminated with HPAI. Our way of including deposition (that is, particle settling and accumulation on the ground) is in contrast to the existing plume models for wind-borne spread of FMDV and allows us to consider infection risks from inhalation by poultry of the originally deposited dust that becomes air-borne due to chicken activity instead of direct inhalation of air-borne dust arriving at ground level. We also include virus decay, as this influences the infection risks arising from deposited dust. Our model framework also allowed us to investigate dust deposition patterns between farms, which is relevant as a possible component of multi-stage indirect transmission mechanisms.

Materials and Methods

In this section, we describe all the processes involved in the wind-borne spread of disease between two poultry farms. We start by modelling particle dispersion and deposition and proceed to determine the quantity of viable virus available in the deposited quantity. We then determine the distance-dependent risk of infection for farms downwind of an infected farm. Lastly, we compare our model estimates for distance-dependent probability of infection with a kernel derived from the Dutch 2003 HPAI epidemic data [10] that presents the averaged distance-dependent probability of infection.

Dispersion model

Dust plume dispersion is assumed to originate from an elevated point source on a poultry house. A model of the motion and deposition of the (contaminated) dust plume is then used to calculate the quantity of viable virus in dust deposited at various locations as the plume moves. This model incorporates particle settling and pathogen decay and the principles of a 3D-Gaussian Plume Model (GPM) and assumes no barriers to the plume. This is a worst-case assumption for the Dutch situation since these barriers would reduce the distance covered by wind-dispersed particles.

The GPM used in this study was obtained by solving a simplified version of the general Advection-Diffusion (A-D) equation (Supporting Information S1). The classic GPM does not consider that during downwind motion the dust particles may settle down due to gravitational and other forces. However, we consider this process to be

essential for two reasons: first, particle settling reduces the amount of dust moving further downwind, and second, we will be interested in the exposure of animals downwind to virus in settled dust. Hence, the first extension we make is incorporating particle settling, at a velocity *v*, into the classic GPM (Supporting Information S1). Particle settling leads to a shift, of magnitude $v\frac{x}{u}$, in the plume centre, where $\frac{x}{u}$ is the duration of plume flight. This gives the adjusted model as

$$C(x,y,z,t)=\frac{Q\left(t-x/u\right)}{2\pi u\sigma_y(x)\sigma_z(x)}\exp\left[-\left(\frac{y^2}{2\sigma_y^2(x)}+\frac{\left(z-\left(H-v\frac{x}{u}\right)\right)^2}{2\sigma_z^2(x)}\right)\right]. \quad (1)$$

Here *H* is the effective release height, *u* is the wind speed, $C(x,y,z,t)$ is the concentration of material at any location (x,y,z) at time *t*, $Q\left(t-x/u\right)$ is the "mass flux" or strength of the emitting source, and $\sigma_y^2=\frac{2K_yx}{u}$ and $\sigma_z^2=\frac{2K_zx}{u}$, where K_y and K_z are respectively the lateral and vertical eddy diffusivities. The factor $\frac{Q\left(t-x/u\right)}{u}$ represents the total cross-sectional amount of dust per meter at a given location a distance *x* away from the source and $\frac{1}{\sqrt{2\pi}\sigma_y(x)}\exp\left[-\left(\frac{y^2}{2\sigma_y^2(x)}\right)\right]$ and $\frac{1}{\sqrt{2\pi}\sigma_z(x)}\exp\left[-\frac{\left(z-\left(H-v\frac{x}{u}\right)\right)^2}{2\sigma_z^2(x)}\right]$ are respectively the lateral and vertical dispersion components. Equation (1) was derived earlier (see Peterson and Lighthart [57] and Lighthart and Mohr [58]) and is used here as a starting point in the development of a calculation of the deposition pattern of the emitted particles.

Deposition model

Particle deposition occurs as a consequence of the vertical plume expansion due to diffusion and particle settling due to gravitation. To model deposition, we first calculate the cumulative quantity deposited per square meter between the source and distance *x*, $D_{\text{cum}}(x,y,t)$ from the difference between the total quantity emitted and the part of the plume that is still air-borne at this point. Mathematically, this quantity is given by integrating the product of the total cross-sectional amount of dust and the vertical dispersion component in equation (1) with respect to *z* from negative infinity up to zero and multiplying it with the lateral dispersion component as

$$D_{\text{cum}}(x,y,t) = D_{\text{cum}}(x,t)\frac{1}{\sqrt{2\pi}\sigma_y(x)}\exp\left[-\left(\frac{y^2}{2\sigma_y^2(x)}\right)\right] \tag{2a}$$

where

$$D_{\text{cum}}(x,t) = \int_{-\infty}^{0}\frac{Q\left(t-x/u\right)}{\sqrt{2\pi}u\sigma_z(x)}\exp\left[-\frac{\left(z-\left(H-v\frac{x}{u}\right)\right)^2}{2\sigma_z^2(x)}\right]dz\,. \tag{2b}$$

The quantity deposited per unit area per second at a specific point at a distance *x* from the source $D(x,y,t)$ is now obtained from equation (2a) by taking the co-moving derivative $u\frac{d}{dx}$ of the cumulative quantity $D_{\text{cum}}(x,t)$ as

$$D(x,y,t) = u\frac{dD_{\text{cum}}(x,t)}{dx}\frac{1}{\sqrt{2\pi}\sigma_y(x)}\exp\left[-\left(\frac{y^2}{2\sigma_y^2(x)}\right)\right]. \tag{3}$$

An alternative way to calculate the total deposited quantity in a GPM, by integrating the vertical diffusion and settling rates of particles at ground level, is described in [59].

We then calculate the total quantity deposited per second on a rectangular area A_h that is *2a* units wide (crosswind direction) and *2b* units long (downwind direction). If this area is always directly under the plume centre (that is, with no change in wind direction during the time of interest), we obtain this quantity by first integrating equation (3) with respect to *y* between the limits $[-a,a]$ and integrate with respect to *x* between the limits $[x-b,x+b]$. If we consider an off-plume-centre location $(r\cos\theta, r\sin\theta)$ at distance *r* from the source farm and at an angle θ with the wind direction, the integration with respect to *x* is between the limits $[r\cos\theta-b, r\cos\theta+b]$ and the one

with respect to *y* is between the limits $\left[r\sin\theta - a, r\sin\theta + a\right]$ that is,

$$\int_{r\sin\theta-a}^{r\sin\theta+a} \frac{1}{\sqrt{2\pi}\sigma_y\left(r\cos\theta\right)} \exp\left[-\left(\frac{y^2}{2\sigma_y^2\left(r\cos\theta\right)}\right)\right] dy\,.$$

Carrying out the lateral integration explicitly yields the expression that estimates the total quantity deposited per second on an area A_h that is *4ab* square units, located at a distance *r* from the source as

$$f\left(r,\theta,t\right) = \int_{r\cos\theta-b}^{r\cos\theta+b} \frac{Q\left(t-x/u\right)}{4x}\left(H+v\frac{x}{u}\right)\frac{1}{\sqrt{2\pi}\sigma_z\left(x\right)} \exp\left(\frac{\left(H-v\frac{x}{u}\right)^2}{2\sigma_z^2\left(x\right)}\right)\left(\mathrm{Erf}\left(\frac{a-r\sin\theta}{\sqrt{2}\sigma_y\left(x\right)}\right)+\mathrm{Erf}\left(\frac{a+r\sin\theta}{\sqrt{2}\sigma_y\left(x\right)}\right)\right)dx. \quad (4)$$

Accumulation and pathogen decay

Consider virus particles emitted in a "puff" spanning a time interval $\left[t_0, t_1\right]$ and decaying exponentially with rate constant λ. The accumulation and decay factor is obtained (see Supporting Information S1) as

$$A\left(t,x\right) = \begin{cases} \frac{1}{\lambda}\left[\exp\left(-\lambda\frac{x}{u}\right) - \exp\left(-\lambda\left(t-t_0\right)\right)\right], t_0 + \frac{x}{u} < t < t_1 + \frac{x}{u} \\ \frac{1}{\lambda}\left[\exp\left(-\lambda\left(t-t_1\right)\right) - \exp\left(-\lambda\left(t-t_0\right)\right)\right], t \geq t_1 + \frac{x}{u} \\ 0, \text{otherwise} \end{cases} . \quad (5)$$

It describes the accumulation of viable pathogen over time and gives the expected proportion of the particles that are still viable at time *t*. It takes into account virus decay during plume flight and while on the ground after deposition and its distance-independence is due to the fact that decay starts as soon as particles are released.

The total contaminated quantity $D_{\mathrm{Total}}\left(r,\theta,t\right)$ available at a given location $\left(r\cos\theta, r\sin\theta\right)$ downwind after time *t* is obtained by taking the product of equations (4) and (5) as

$$D_{\mathrm{Total}}\left(r,\theta,t\right) = A\left(r,t\right) f\left(r,\theta,t\right). \quad (6)$$

Equation (6) defines the model for our study. In order to make a direct comparison of our predictions with the result of Boender et al. [10] which is a kernel describing the distance-dependence of transmission risk (averaged over all directions), we integrate

the deposition function over all possible downwind directions and normalize the outcome. This gives the average contaminated quantity deposited as

$$D_{\text{Average}}(r,t) = \frac{1}{\pi}\int_{-\pi/2}^{\pi/2} D_{\text{Total}}(r,\theta,t)\,d\theta\,. \tag{7}$$

This yields a fairly complex expression and thus the analytical insight obtained from it is limited. Therefore, most of the results discussed below are obtained by numerical analysis.

One question of interest is the distance from the source to the point of maximum deposition. This distance is calculated by solving the equation $\frac{dD_{\text{Average}}(r,t)}{dr} = 0$ for *r*, which again gives a complicated expression. Hence a numerical exploration of the effects of varying the model parameters is performed in the sensitivity analysis (Supporting Information S1).

Estimating the distance-dependent infection risk for the receiving farms

Virus amount and infection probability models

To translate the predicted deposition of dust into virus amount we use results reported by Shortridge et al. [60] for the virus titer τ_v in originally wet faeces held at 25°C for 4 days. The log-transformed virus amount in w grams, $\tau(w)$ is given by

$$\tau(w) = \tau_v + \log_{10} w \tag{8}$$

in units of $\log_{10}\text{EID}_{50}$. Subsequently, we determine the probability of infection of a chicken for a given virus amount inhaled based on a dose-response curve that we obtain by fitting to experimental data of Spekreijse et al.[61]. We use a dose-response function (probability of infection as a function of dose) as derived by Lange and Ferguson [62] based on assuming that there is a finite probability of infection for any virus amount even though the probability decays exponentially fast with reducing virus amount. For an inhalation involving w grams, the probability of infection $p(w)$ is given by

$$p(w) = \frac{1}{1+\exp(\alpha+\gamma\tau(w))}\,. \tag{9}$$

where α and γ are the shape parameters for the fitted logistic curve. This dose-response function is consistent with the Independent Action Hypothesis [63].

The inhalation model

Chicken activities such as pecking, wing flapping, dust bathing and other movements suspend the already settled virus particles that they subsequently inhale. A study on determining the lung volume of chickens [64] reports a volume of $1.4\times10^{-5}\,\mathrm{m}^3$ for a 24 days-old broiler chicken (which gives the limiting air sampling capacity $\left(V_{\max}\right)$ used in this study) and another study to determine the respiratory rate of chickens [65] reports a range of 27 to 31 min^{-1}. Furthermore, since the components of farm dust which include faeces, skin and feathers, bedding material and feed-remains are not equally infectious, part of this material acts merely as a vector onto which the infectious part colloids during dispersal. The contaminated fraction $\left(F_c\right)$ is taken to be 10% which is the relative amount of excreta in the litter attributable to chicken droppings [66]. More to that, the contaminated dust originating from infected premises is diluted upon mixing with (initially uncontaminated) resident dust. The resulting composition of the dust to be inhaled is determined by scaling the quantity of the incoming contaminated dust by the amount of resident dust per unit area in a poultry house $\left(D_{\text{Resident}}\right)$ to obtain the fraction of contaminated dust in the total whirled-up dust. The concentration of inhaled dust is estimated by multiplying an estimate of the average dust concentration in a poultry house $\left(C\right)$ with a concentration ratio *c* describing how much the average concentration is exceeded closely above the ground. We use the average of the ratios of dust concentrations at 40 cm and 260 cm from [67]. Combining these model elements gives the weight of infectious material w_I inhaled per inhalation as

$$w_I = \left(\frac{D_{\text{Average}}\left(r,t\right)}{D_{\text{Resident}}}\right)\times V_{\max}\times F_c\times c\times C\,. \tag{10}$$

The within-flock epidemic model

Upon intake of the virus, infection may or may not occur depending on the virus amount inhaled. Given a successful first infection, subsequent infections at the farm level may occur, resulting into a major outbreak on the farm. In this study, infection risk is defined as the ability of the deposited virus to cause an infection of at least one susceptible bird in a flock and this bird being able to set off a major within-flock epidemic. For a flock with *N* birds, the hourly probability $p_k\left(t\right)$ of infecting *k* birds is given by

$$p_k\left(t\right) = \sum_{k=1}^{i}\binom{N}{k}\left(1-p\right)^{(N-k)f}\left(1-\left(1-p\right)^{f}\right)^{k}, \tag{11}$$

where p is the probability of infection per inhalation defined using equation (9) as $p = p(w_I)$.

For a disease which has a within-flock basic reproduction ratio R_0 (defined as the number of secondary infections caused by a primary case in an entirely susceptible population), where for $R_0 > 1$, a major outbreak can occur, otherwise only a minor outbreak can occur [68], the probability of a major outbreak within the flock given *k* initial infections is $1-\left(\frac{1}{R_0}\right)^k$. Therefore, the overall probability of infection of the flock P_{Final} can be obtained from the product of the probability of having *k* initial infections and the probability that these infections cause a major within-flock epidemic as

$$P_{\text{Final}} = 1-\prod_{t=1}^{\infty}\left(1-\sum_{k=1}^{i} p_k(t)\left(1-\left(\frac{1}{R_0}\right)^k\right)\right). \quad (12)$$

We note that, unlike the deposition pattern, the probability of infection does not depend on available chicken space A_h. Rather, as described by equation (10), it is limited by the sampling capacity $V_{\max}$ of the chicken.

Assessing the contribution of the wind-borne route

The contribution of the wind-borne route to the epidemic is here determined by the fraction of new cases that it can explain. We use the concept of the between-farm (basic) reproduction ratio (as defined in the Supporting Information S1) to compute this fraction.

Estimates for the parameters applicable to the HPAI situation

General and HPAI-specific parameter estimates are used in this study to quantitatively assess the possible role of the wind-borne route in the indirect transmission of the virus. They are categorised into dispersion, pathogen, host and farm related parameters.

Dispersion-related parameters include the emission quantity $Q\left(\text{gs}^{-1}\right)$ (which depends on the number birds on the farm, concentration of dust $C\left(\text{gm}^{-3}\right)$ and ventilation rate $\left(\text{gs}^{-1}\right)$), particle settling velocity $v\left(\text{ms}^{-1}\right)$, effective release height

$H\left(\mathrm{m}\right)$, wind speed $u\left(\mathrm{ms}^{-1}\right)$, vertical and lateral eddy diffusivity $K_z\left(\mathrm{m}^2\mathrm{s}^{-1}\right)$ and $K_y\left(\mathrm{m}^2\mathrm{s}^{-1}\right)$ respectively. The total dust emission rate (both inhalable and respirable), taken from Takai et al. [69] is $0.0122\,\mathrm{ghr}^{-1}$ per bird and the total dust concentration is $0.0052\,\mathrm{gm}^{-3}$. The average settling velocity for broiler house particles reported by Gustafsson and Mårtensson [70] is approximately $0.01\mathrm{ms}^{-1}$. The effective release height was estimated as 6 m, based on the poultry house height of 5 m [67] and assuming an initial plume rise due to buoyancy of 1 m. According to Berge et al. [71], the vertical eddy diffusivity for outdoor plume modelling is $0.03\mathrm{m}^2\mathrm{s}^{-1}$.

The pathogen-specific parameters are the decay rate constant $\lambda\left(\mathrm{s}^{-1}\right)$ and dose-response parameters which depend on the combination of pathogen- and host-specific characteristics. For a virus that survives for 4 days [60,72], the decay rate constant is calculated to be $2.89\times10^{-6}\mathrm{s}^{-1}$. The host-specific parameters include the number of breathes per chicken per hour $\left(f\right)$ and the parameters that (through equation (9)) determine the probability of infection given an inhalation $\left(p\right)$. The farm-specific parameters include the flock size and the basic reproduction ratio $\left(R_0\right)$. The estimate for the transmission rate parameter $\left(\beta\right)$ that Bos et al. [73] obtained using Dutch 2003 epidemic data is 4.5 per infectious chicken per day. To estimate the chicken infectious period T_{inf}, they used data from an experiment in which 7 out of 10 chickens died (resulting into $T_{\mathrm{inf}}=4$ days), and the remaining 3 survived till the end of the experiment, here taken as 7.5 days that is, the average time between infection and depopulation during the outbreak [10]. In this study, these two pieces of information are combined to obtain a weighted average for the infectious period of 5.05 days and consequently, a within-flock R_0 for the H7N7 HPAI strain of 22.7. For other strains of the virus, R_0 may be smaller, for example, it is estimated to be between 2.2 and 3.2 for the H5N1 HPAI strain [35]. Based on 7-days mortality data used in [73] and using a simple SIR model for within-flock transmission, we estimate that the reported mortality would correspond to an average number of infectious birds per day in a flock of roughly 100. To estimate the prevailing wind speed, we used data recorded at three weather stations (two in the central and one in the southern part) in the Netherlands during the epidemic (the period between February 28th and May 31st 2003) (available on the website: http://www.knmi.nl/klimatologie/daggegevens/selectie.cgi). From the downloaded data, we calculated the average (minimum-maximum) wind speed during

the outbreak as $3.7(1.0-8.5)\,\mathrm{ms}^{-1}$. Here we use the average and perform a sensitivity analysis over the whole range (Supporting Information S1). A summary of all the parameter estimates is given in Table 1.

Table 1. Default parameter values used in the model calculations

Parameter	Value	Source
Total dust emission rate, Q	0.0122 g hr^{-1} per bird	Takai et al. [69]
Total dust concentration, C	0.0052 gm^{-3}	Takai et al. [69]
Concentration ratio, c	1.03*	Yushu and Baoming [67]
Log-transformed virus titer, τ_v	$1.5\ \log_{10}\mathrm{EID}_{50}/\mathrm{g}$	Shortridge et al. [60]
Particle settling velocity, v	0.01 ms^{-1}	Gustafsson and Mårtensson [70]; Hinds [74]
Decay rate constant, λ	2.89×10^{-6} s^{-1}	Webster et al. [72]; Shortridge et al. [60]
Wind speed, u	3.7 ms^{-1}*	Meteorological data (KNMI)
Flock size, N	10,000*	Thomas et al. [22]
Effective release height, H	6 m*	Yushu and Baoming [67]
Eddy diffusivities, K_z and K_y	0.03 m^2s^{-1}	Berge et al. [71]
Infection rate per day, β	4.5 day^{-1}	Bos et al. [73]
Weighted infectious period, T	5.05 days*	Bos et al. [73]
Basic reproduction ratio, R_0	22.7*	Bos et al. [73]
Dose-response curve parameters α and γ	4.76 and – 1.87*	Spekreijse et al. [75]
Area per hen (free range), $A_h = 4ab$	4 m^2	EC [76]
Sampling capacity, V_{max}	1.4×10^{-5} m^3	Julian [64]
Contaminated fraction, F_c	10%	Koerkamp et al. [66]
Inhalations per hour, f	1.62×10^3*	Pampori and Iqbal [65]
Resident dust amount per day, $D_{Resident}$	1.97 gm^{-2}*	Gustafsson and von Wachenfelt [77]

*****parameter value estimated from the data in the indicated reference

Results

The model predictions presented here were obtained using the parameters given in Table 1 and the models given by equations (7 - 12). We present the model-predicted deposition pattern for contaminated dust in Figure 1, and in Figure 2 we show the comparison between the distance-dependent probability of infection as estimated by Boender et al. [10] from the 2003 epidemic data and our wind-borne spread model prediction. The fraction of new cases caused by the wind-borne route up until a given distance $r_{\text{cut-off}}$ during the epidemic is presented in Figure 3. It is calculated for various choices of the cut-off distance $r_{\text{cut-off}}$. In the Supporting Information S1, we present

detailed sensitivity analyses of the effect, on the deposition pattern, of varying; the wind speed (Figure S1), settling velocity (Figure S2), eddy diffusivity (Figure S3), effective release height (Figure S4), and decay rate (Figure S5). In Figure S6, we present the effect of varying the decay rate, the settling velocity and the within-flock basic reproduction ratio on the distance-dependent probability of infection.

The predicted dispersal pattern of HPAI virus on dust

Following wind-borne dispersal of contaminated farm dust, we calculated the quantity of contaminated dust present on a given space (area per hen, $A_h = 4ab$) on an outdoor run of a farm. The predicted deposition pattern after a 24 hour-long emission is presented in Figure 1.

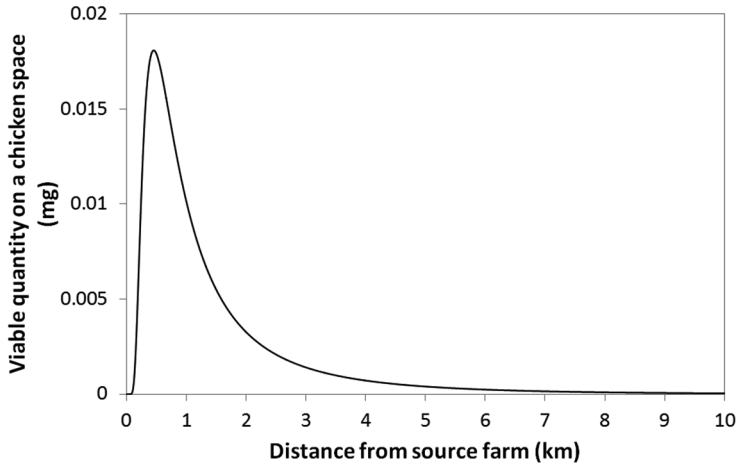


Figure 1. Contaminated dust quantity present on a 4 m square space at various distances from the source for the parameter values given in Table 1 at the moment that the deposition arising from a 24 hour-long emission period ends.

We observe (Figure 1) that for our choice of parameter values, there were no substantial quantities of contaminated dust present at distances less than 0.05 km from the source. This is because the model assumes that the particles are released through a raised vent (5 m above ground level). Beyond 0.05 km, the contaminated quantity present at a given location increased to its maximum at approximately 0.45 km from the source after which it starts to decrease. We use this result to estimate the distance-dependent risk of infection associated with the contaminated quantity present at a given location and compare the outcome with observed epidemic transmission pattern.

Comparison with Dutch 2003 HPAI epidemic pattern

We calculate the distance-dependent probability of infection for farms downwind of an infected farm by combining our model predictions of the hourly depositions with the virus amount and infection probability models, the inhalation model and the within-flock epidemic model as described in the Materials and Methods section. We use the Dutch 2003 epidemic data to test whether wind-borne HPAI spread was possible and if so, determine its possible contribution during the epidemic by comparing our model predictions with the observed pattern in the epidemic. As can be seen from equations (7-12), for small infection probability per inhalation the model-predicted probabilities are to a very good approximation proportional to the deposition pattern (as given by equation (7)). As a result, in the parameter range of interest here, the distance-dependence of the model-predicted probabilities is practically indistinguishable from that of the deposition pattern.

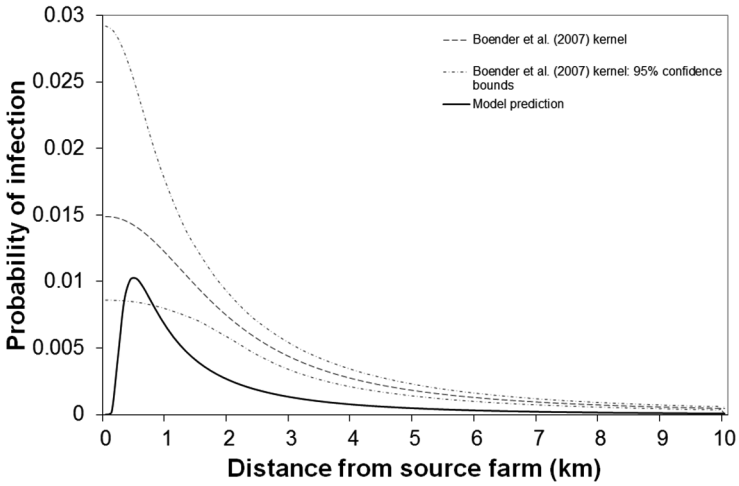


Figure 2. The distance-dependent probability of infection for the parameter values given in Table 1 and the Boender et al. (2007) transmission kernel (and its 95% confidence bounds). The calculation caters for the prolonged infectiousness of the wind-dispersed material beyond the (direct-contact) infectious period of the source farm.

The comparison in Figure 2 more importantly shows a qualitative difference in the tail. Compared to the observed pattern, there is a faster drop in the predicted infection probability beyond 0.45 km. At all distances from the source, the predicted probabilities are smaller than the observed risk. Also, beyond 1 km distance the predicted risk of solo wind-borne infection is decaying significantly faster with distance than the observed risk. The observed rapid decrease of the predicted risk with distance (Figure 2) is only very weakly sensitive to the precise value of pathogen decay rate, settling velocity and the within-flock basic reproduction ratio as shown in Figure S6. Based on these results, we conclude that the wind-borne route alone could not explain the pattern of the 2003 epidemic.

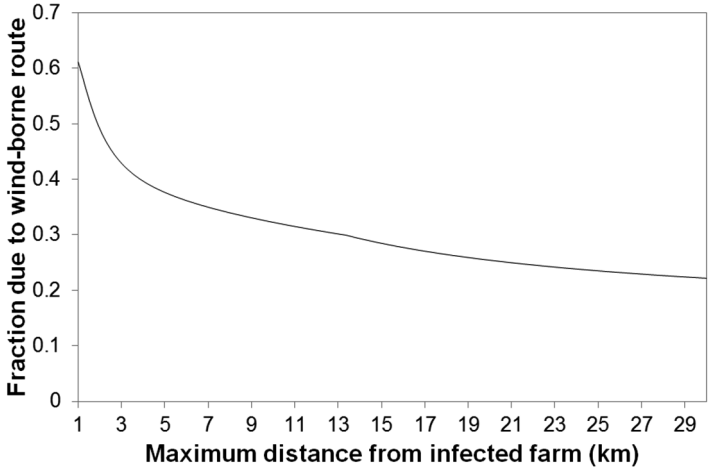


Figure 3. The fraction of the total number of new infections as estimated by Boender et al. (2007) from the 2003 epidemic data attributable to the wind-borne route for various choices of a cut-off distance up until which the new infections are occurring.

Figure 3 shows that the fraction of new cases that could be solely attributed to the wind-borne route decreases with increasing cut-off value $r_{\text{cut-off}}$. We consider the distance range of $r_{\text{cut-off}} = 25$ km to be most relevant as it corresponds to the width of the poultry-dense area in which the 2003 outbreak started [10]. Within this distance range, we estimate that the wind-borne route on its own could explain up to 24% of the new cases. Consequently, we conclude that the wind-borne route may have played a significant role in the spread of HPAI during the Dutch 2003 epidemic although it was not the only transmission route.

Discussion

Quantification of the dispersal pattern of contaminated farm dust is of great importance in developing an understanding of the indirect transmission of livestock diseases between farms. In this paper, the quantity of viable virus deposited at locations downwind of a source farm is calculated using a GPM, and the significance of various model parameters to the deposition pattern is assessed. Based on our model predictions in the context of the spread of HPAI, the wind-borne route alone is insufficient to explain the observed pattern during the 2003 epidemic in the Netherlands. In particular, although it could have played a significant role in the shorter distance transmission events, it cannot explain the long-range transmission probabilities estimated in [10] from the observations in 2003. The calculation of the contaminated dust quantity deposited between farms could be a starting point for studies on multi-stage indirect transmission through a combination of different routes.

This modelling framework can also be used to study the wind-borne spread of other pathogens.

In the sensitivity analysis (Supporting Information S1), we analysed the effects of varying wind speed (Figure S1), settling velocity (Figures S2 and S6), vertical eddy diffusivity (Figure S3), effective release height (Figure S4), the decay rate (Figures S5 and S6) and the within-flock basic reproduction ratio (Figure S6). The parameters to be explored were chosen based either on their importance to the dispersion process or on the uncertainty in estimating their values. A further reason for selecting the settling velocity and decay rate was to elucidate their importance in the study of wind-borne spread of livestock diseases, given that they are often neglected, for example in plume model studies of wind-borne spread of FMDV. The results of these analyses (Supporting Information S1) reveal the robustness of our main result. In other words, the discrepancy at farther away distances of the predicted risk and that observed during the epidemic as depicted in Figure 2 for the default parameter values of Table 1 holds for all ranges of parameter values explored. This is because, for all explorations, the resulting kernels have thinner tails compared to the pattern of the Dutch 2003 epidemic.

Since we were interested in assessing the role of wind-borne spread during the Dutch 2003 epidemic that involved an H7N7 HPAI subtype, we chose a within-flock basic reproduction ratio $\left(R_0\right)$ specific to this strain. However, for other strains such as the H5N1, the corresponding R_0 is smaller that is, in the range of 2.2 to 3.2 [35], and this consequently reduces the probability of a major within-flock outbreak although it is within the same order of magnitude as that predicted for the H7N7 HPAI virus strain considered in this study. Hence, we conclude that the predicted risk of infection by other virus strains at farther away locations will ultimately follow the same pattern as that of the H7N7 HPAI strain. Due to the lack of data on dose response and virus shedding for the H7N7 HPAI strain, we used data on H5N1 HPAI strain. However, the sensitivity analyses performed revealed that changes in these parameters do not alter the main conclusion of this study.

We conclude that the wind-borne route cannot fully explain observed patterns of between-farm spread of the virus especially for longer distances. This conclusion is robust to changes in uncertain model parameters. We also estimate that, up until 25 km distance, wind-borne transmission could explain up to 24% of the observed infections. This latter percentage is subject to some uncertainty. Nevertheless, this result supports the need to identify supplementary mechanisms that aid the transportation of the virus between locations. It also implies that: a) the experienced neighbourhood transmission was not entirely due to wind dispersal of the virus, b) virus transportation may either have entirely been by a different mechanism in a single-stage process, or c) virus transportation may have been by a multi-stage process that also involves the wind dispersal. Consequently, in-depth studies on the role of fomites in the transfer of infectious material between flocks are essential to develop alternative models for indirect transmission.

The deposition modelling approach developed here is likely to be relevant to modelling of wind-borne spread of other livestock diseases as well. Particles to which pathogens may be attached in wind-borne dispersal, have a size range of 1 to 100 μm and they sediment under gravity [74,78,79]. Therefore, it seems unrealistic to neglect the effect of deposition on the risk of wind-borne spread of livestock diseases. Also, it is important to incorporate pathogen decay when studying the wind-borne virus spread, especially for spread over more than just a few kilometres. For the case of FMDV this has previously been shown by Hess and others [80]. We have found, in the sensitivity analysis, that both deposition and pathogen decay have a significant effect on the ground level air-borne dust concentration at larger distances from the source (Supporting Information S1). These findings illustrate the general importance of considering the survival characteristics of the virus strain involved as well as the process of particle settling during plume motion if a reliable assessment of the risk of wind-borne spread of the livestock diseases is to be made.

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Supporting Information

1. Derivation of the Gaussian Plume Model

The GPM used in this study was obtained by solving a simplified version of the general Advection-Diffusion (A-D) equation given by

$$\frac{\partial C}{\partial t} = \sum_i \left(u_i \frac{\partial C}{\partial x_i} - K_i \frac{\partial^2 C}{\partial x_i^2} \right) = 0,$$

where $i = (x, y, z)$ are Cartesian point coordinates $u_i = (u_x, u_y, u_z)$, where $u_i \neq 0$, is the wind speed vector, $K_i = (K_x, K_y, K_z)$ is the eddy diffusivity vector, and *C* is the concentration of material at any location (x, y, z) at time *t*. Assuming that the wind direction is along the *x* axis and denoting $u_x = u$ and that the advection in the downwind direction is overwhelmingly large compared to the turbulent diffusion in the same direction, that is,

$$u \frac{\partial C}{\partial x} \gg K_x \frac{\partial^2 C}{\partial x^2},$$

and considering the steady state solution $\frac{\partial C}{\partial t} = 0$ since the concentration at time *t* and a distance *x* downwind from the source is proportional to the source strength at the time $\left(t - \frac{x}{u} \right)$, the A-D equation simplifies to the second-order parabolic partial differential equation

$$u \frac{\partial C}{\partial x} - K_y \frac{\partial^2 C}{\partial y^2} - K_z \frac{\partial^2 C}{\partial z^2} = 0.$$

As Hunter et al. [81] showed, for constant eddy diffusivities, Taylor series expansions of K_y and K_z about the origin lead to variances that increase at rates equal to twice the eddy diffusivities as $\sigma_y^2 = \frac{2K_y x}{u}$ and $\sigma_z^2 = \frac{2K_z x}{u}$.

The classic GPM is a particular solution to an A-D equation under the assumption of constant eddy diffusivities. This particular solution, which defines the GPM model, is given by

$$C(x, y, z, t) = \frac{Q\left(t - x/u\right)}{2\pi u \sigma_y(x) \sigma_z(x)} exp\left[-\left(\frac{y^2}{2\sigma_y^2(x)} + \frac{(z - H)^2}{2\sigma_z^2(x)} \right) \right]. \qquad \text{(S1)}$$

Equation (S1) applies to emissions from an elevated point source at $(0, 0, H)$, where *H* is the effective release height given by the sum of a height *h* and an initial plume rise Δh due to buoyancy.

Some authors, for example Gloster and others[53] consider ground level release of particles (i.e., $H=0$) and are only interested in the Ground Level Concentration $\left(C_{gl}\right)$ of particles (i.e. when $z=0$). Thus their model takes the form

$$C\left(x,y,0,t\right)=\frac{Q\left(t-x/u\right)}{2\pi u\sigma_y\left(x\right)\sigma_z\left(x\right)}exp\left[-\frac{y^2}{2\sigma_y^2\left(x\right)}\right]. \tag{S2}$$

2. Deriving the accumulation and decay function

Consider virus particles emitted in a "puff" spanning a time interval $\left[t_0,t_1\right]$ and decaying exponentially with rate constant λ. For a unit release per second, the amount of viable virus at time *t* is given by the convolution of the emission and decay functions as

$$\int_{-\infty}^{t}H\left(\tilde{t}-\left(t_0+\frac{x}{u}\right)\right)H\left(t_1+\frac{x}{u}-\tilde{t}\right)exp\left(-\lambda\left(\frac{x}{u}+t-\tilde{t}\right)\right)d\tilde{t}\ . \tag{S3}$$

Where *H(t)* denotes the Heaviside function, and $\tilde{t},t\ \text{and}\ \frac{x}{u}$ are respectively the time of deposition, time of interest and plume flight-time until locations $\left(x,y,z\right)$. Applying the definition of *H(t)* and carrying out the integration in (S3) yields the accumulation and decay factor as

$$A\left(t,x\right)=\begin{cases}\frac{1}{\lambda}\left[\exp\left(-\lambda\frac{x}{u}\right)-\exp\left(-\lambda\left(t-t_0\right)\right)\right], t_0+\frac{x}{u}<t<t_1+\frac{x}{u}\\ \frac{1}{\lambda}\left[\exp\left(-\lambda\left(t-t_1\right)\right)-\exp\left(-\lambda\left(t-t_0\right)\right)\right], t\geq t_1+\frac{x}{u}\\ 0,\text{otherwise}\end{cases}\ . \tag{S4}$$

3. The between-farm (basic) reproduction ratio and the contribution of wind-borne route

The between-farm (basic) reproduction ratio *(R)* is defined as the expected number of secondary infections caused by one infected farm in a totally susceptible population of surrounding farms. For a constant farm density ρ, it is given by $R=2\pi\rho\int_0^\infty p\left(r\right)rdr,$ where $p\left(r\right)=1-exp(-h\left(r\right)T)$ and *T* is the infectious period of the farm (set at 7.5 days) and *h(r)* is the transmission kernel. We use this formulation to calculate the fraction

of new infections attributable to the wind-borne route for the infections occurring up until a distance $r_{\text{cut-off}}$ from

$$\frac{\int_0^{r_{\text{cut-off}}} P_{\text{Final}} r dr}{\int_0^{r_{\text{cut-off}}} p(r) r dr},$$

where P_{Final} is given in equation (12) (main text). We explore a number of values for $r_{\text{cut-off}}$ up to 30 km in Figure 3 (main text).

4. Sensitivity Analysis

In these sensitivity analyses we concentrate on the viable contaminated dust quantity present at a given location at the moment that the deposition arising from a 24-hour emission period ends. The intrinsic multiplicative character of exposure and dose response obtained in the derivation of the probability of infection (equation (12) in the main text) guarantees the proportionality of the effect of parameter changes on the model predictions for the probability of infection and the quantity deposited. The graphs present the viable quantity available on a 4 m^2 area at the moment that the deposition arising from a 24 hour-long emission period ends.

Wind speed effect

The predicted deposition patterns for a range of wind speeds (1-8 ms^{-1}) are presented in Figure S1. This range is chosen to cover a whole range of plausible wind speeds that prevailed during the 2003 epidemic available at http://www.knmi.nl/klimatologie/daggegevens/selectie.cgi.

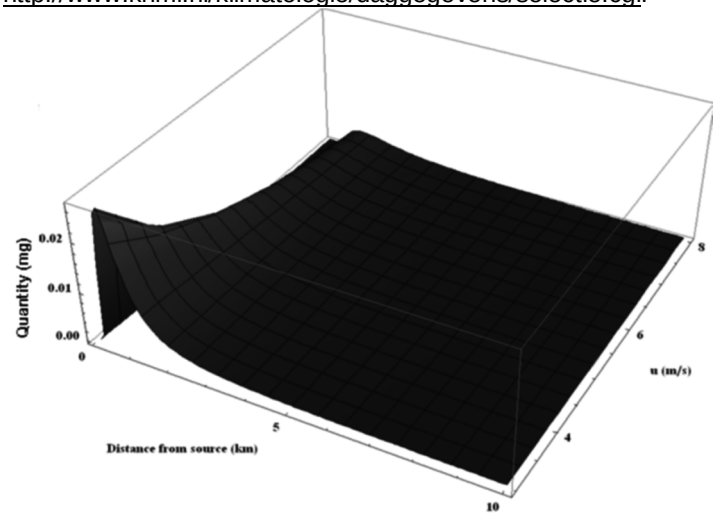


Figure S1. Effect of varying wind speed *u* on the contaminated dust quantity present on a 4 m square space at various distances from the source at the moment that the deposition arising from a 24 hour-long emission period ends.

As the prevailing wind speed increases, the maximum viable quantity present declines and there is a shift, away from the source farm, of the point where it occurs (Figure S1). Across the whole range of wind speeds explored, the deposition pattern still qualitatively conforms to that depicted in Figure 1(main text), which differs from the observed pattern of the epidemic.

Particle settling velocity effect

The settling of the particles in a plume is partially as a result of gravitational pull. When the force of gravity on the particle is greater than the attraction between the particle and the surrounding air molecules, they will sediment [82,83]. The effect of varying this parameter in the range 0.0002 ms^{-1} to 0.017 ms^{-1} is explored in Figure S2. This range is in agreement with the values reported in [70,74].

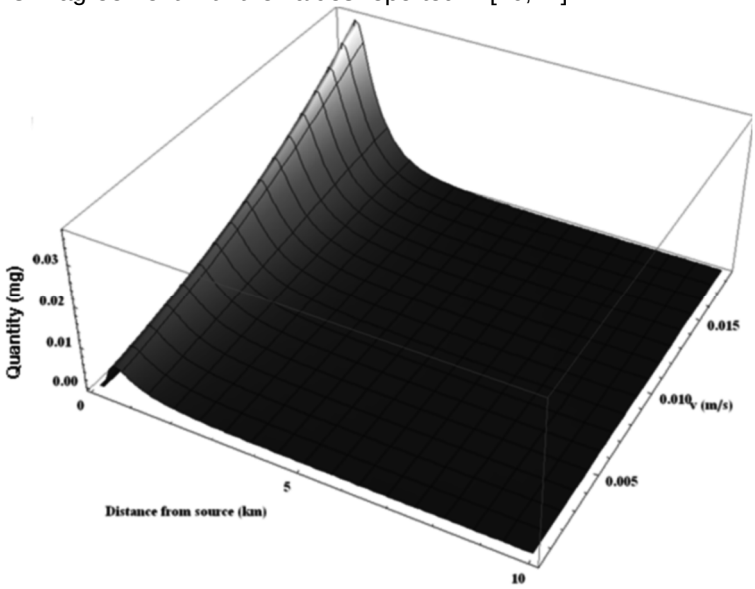


Figure S2. Effect of varying the settling velocity *v* on the contaminated dust quantity present on a 4 m square space at various distances from the source at the moment that the deposition arising from a 24 hour-long emission period ends.

As the settling velocity increases, the dispersal range decreases and the maximum quantity present increases. In other words, particles with a higher settling velocity (e.g., heavier particles) tend to be deposited closer to the source than those with a lower velocity which can be airborne for a longer time. The interaction between the settling velocity and the point of maximum deposition is non-linear, which agrees with the results of Näslund and Thaning [84] and Tellier [85]. For the whole range of the explored parameter values, the feature of a very thin large-distance tail is preserved; hence our conclusion that the wind-borne route is insufficient to explain the observed epidemic pattern holds for all choices of the settling velocity explored.

Vertical eddy diffusivity effect

The rate of growth of the plume surface area due the movement of particles away from the plume centre is defined by the Eddy diffusivity. The effect of varying vertical eddy diffusivity is explored in Figure S3 for the range $8.33\times10^{-4}\,\mathrm{m^2s^{-1}}$ to $1.83\times10^{-1}\,\mathrm{m^2s^{-1}}$; the reported range for indoor and outdoor particle diffusion [71].

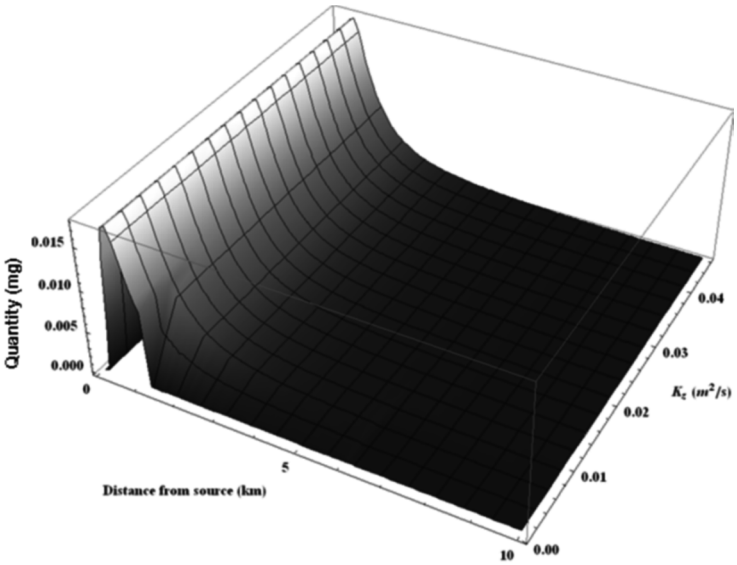


Figure S3. The effect of varying the vertical eddy diffusivity K_z on the contaminated dust quantity present on a 4 m square space at various distances from the source at the moment that the deposition arising from a 24 hour-long emission period ends.

The vertical eddy diffusivity governs the vertical spread-out of the plume. In a stable atmosphere (i.e., small K_z), particles remain airborne for a longer time and the plume is narrower than in an unstable environment. In our model calculations, the effect of this parameter seems insignificant across the range of explored values (Figure S3). Therefore, our result that the infection risk predicted by the model for locations farther away from the source farm is much lower that the observed risk during the 2003 epidemic is robust to changes in the vertical eddy diffusivity.

Effective release height effect

Results for the effect of varying the effective release height in the range 4 to 8 m are presented in Figure S4.

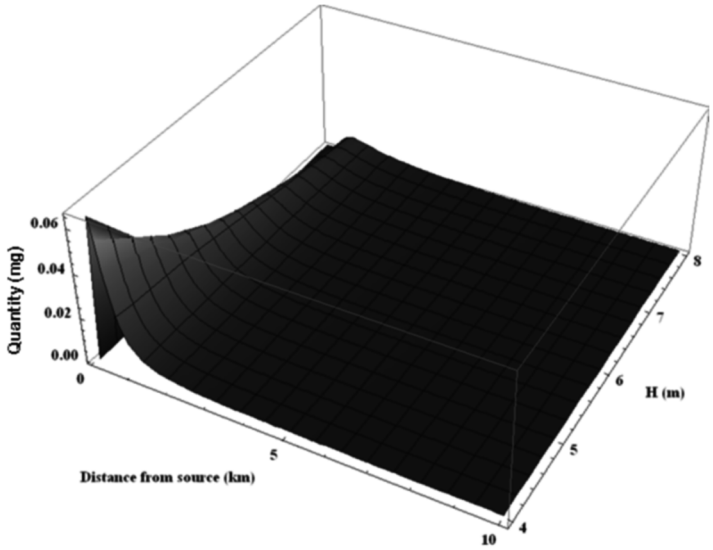


Figure S4. Effect of varying the effective release height *H* on the contaminated dust quantity present on a 4 m square space at various distances from the source at the moment that the deposition arising from a 24 hour-long emission period ends.

There is a non-linear relationship between the effective release height and distance from the source to the point of maximum deposition. For higher release heights, particles remain airborne for a longer time and end up being deposited farther away from the source than those released near the ground surface. For all parameter values explored in Figure S4, the predicted risk of infection is smaller than that observed during the epidemic at farther distances from the source.

Decay rate effect

In Figure S5, we present the results on the effects of varying the decay rate in the range $3.86\times10^{-7}\,s^{-1}$ to $1.653\times10^{-6}\,s^{-1}$ (i.e., virus survival ranging from 4 to 30 days)[72].

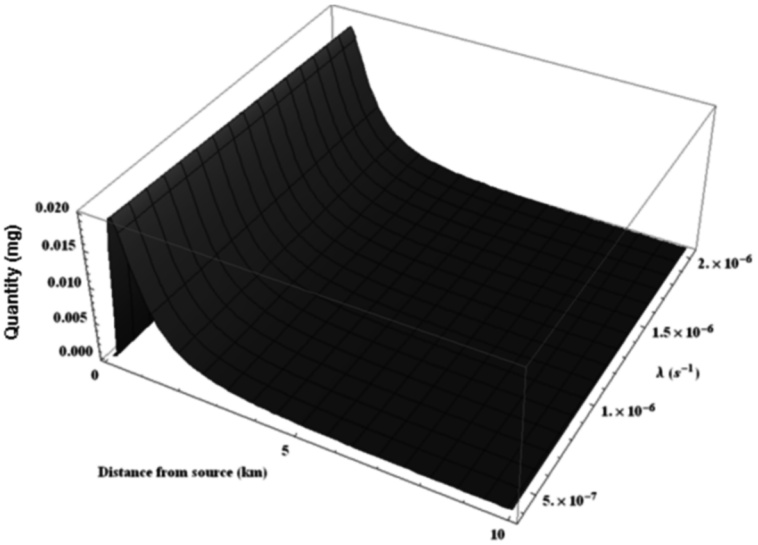


Figure S5. Effect of varying the decay rate λ on the contaminated dust quantity present on a 4 m square space at various distances from the source at the moment that the deposition arising from a 24 hour-long emission period ends.

The point of maximum deposition is the same in all cases but the viable quantity varies: for example, the peak amounts of viable contaminated dust quantities present after a 24-hour long emission are 0.018 and 0.02 mg for 4 and 30 days virus survival respectively.

Effect of the pathogen decay rate, the particle settling velocity and the within-flock basic reproduction ratio on distance-dependent probability of infection

Figures S5 and S2 show the sensitivity of the predicted deposition pattern to the pathogen decay rate and to the particle settling velocity respectively. In Figure S6A-B we indicate how this sensitivity translates to the model-predicted probabilities, and to the comparison of these to the kernel estimated from the 2003 epidemic. In Figure S6C we consider the sensitivity to the within-flock basic reproduction ratio.

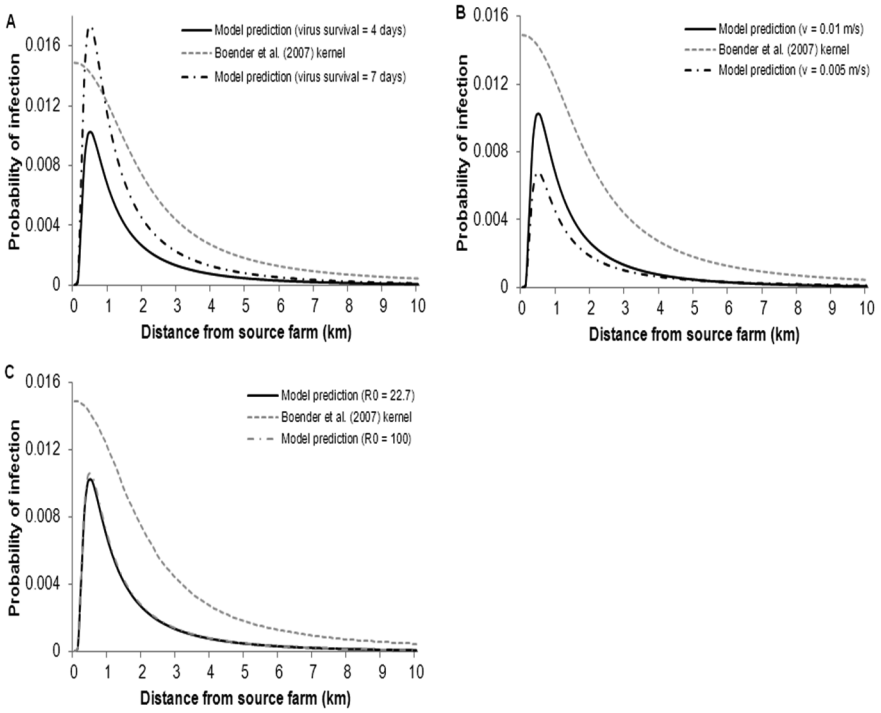


Figure S6. Comparison of the distance-dependent probability of infection as estimated by Boender et al. (2007) from the 2003 epidemic data and our wind-borne spread model prediction with default parameter values and: Panel A. The virus survival was increased from 4 to 7 days); Panel B. The particle settling velocity was reduced from 0.01 m/s to 0.005 m/s); Panel C. The within-flock basic reproduction ratio was increased from 22.7 to 100.

The difference in pattern between the different values of the decay rate observed in panel A of Figure S6 illustrates the importance of including this parameter in modelling wind-borne transmission (as has been discussed before in the context of FMDV by Hess et al.[86]). We observe in panel B that the model prediction is slightly sensitive to the particle settling velocity at shorter distance ranges and in panel C, it is only very weakly sensitive to the within-flock basic reproduction ratio at all distance ranges. At long distances, the model prediction is very weakly sensitive to all the three parameters. The long distance behaviour is consistently lower than observed pattern during the outbreak in all the three cases. This implies that the possible inaccuracies in estimating the decay rate, settling velocity and R_0 have do not affect the main conclusion of this study.

Chapter 3

Estimating the per-contact probability of infection by highly pathogenic avian influenza (H7N7) virus during the 2003 epidemic in the Netherlands

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Abstract

Estimates of the per-contact probability of transmission between farms of Highly Pathogenic Avian Influenza virus of H7N7 subtype during the 2003 epidemic in the Netherlands are important for the design of better control and biosecurity strategies. We used standardized data collected during the epidemic and a model to extract data for untraced contacts based on the daily number of infectious farms within a given distance of a susceptible farm. With these data, we used a maximum likelihood estimation approach to estimate the transmission probabilities by the individual contact types, both traced and untraced. The estimated conditional probabilities, conditional on the contact originating from an infectious farm, of virus transmission were: 0.000057 per infectious farm within 1 km per day, 0.000413 per infectious farm between 1 and 3 km per day, 0.0000895 per infectious farm between 3 and 10 km per day, 0.0011 per crisis organisation contact, 0.0414 per feed delivery contact, 0.308 per egg transport contact, 0.133 per other-professional contact and, 0.246 per rendering contact. We validate these outcomes against literature data on virus genetic sequences for outbreak farms. These estimates can be used to inform further studies on the role that improved biosecurity between contacts and/or contact frequency reduction can play in eliminating between-farm spread of the virus during future epidemics. The findings also highlight the need to; 1) understand the routes underlying the infections without traced contacts and, 2) to review whether the contact-tracing protocol is exhaustive in relation to all the farm's day-to-day activities and practices.

Keywords: highly pathogenic avian influenza virus, H7N7, probability of virus transmission, between-farm contacts, modelling, maximum likelihood

Introduction

Highly Pathogenic Avian Influenza (HPAI) is one of the OIE listed poultry diseases. Several epidemics involving these viruses have occurred world-wide since its first description in northern Italy in 1878 [9,87]. Examples of epidemics with devastating socio-economic consequences are the 1999 H7N1 epidemic in Italy [6] and the 2003 H7N7 epidemic in the Netherlands [8,88]. Consequences of these epidemics include economic losses incurred in implementing control strategies and reduction in exports as well as a risk of spread to humans [7,89]. The HPAI (H7N7) 2003 epidemic in the Netherlands involved 255 flocks; the virus was isolated in 241 of these flocks while the other 14 flocks were serologically positive [8,88]. The majority of affected flocks were located in either of two areas with high poultry farm densities: one comparatively large area situated in the centre of the country, and one smaller area in the south; for more details we refer to Boender et al. [10].

Following the detection of the first outbreak, a control programme, as stipulated by the European Union, was implemented. This programme consisted of stamping out of infected flocks, movement restrictions and establishment of protection and surveillance

zones. Despite additional control measures such as pre-emptive culling of flocks within a radius of 1 km of an outbreak and establishment of buffer zones between defined areas by complete depopulation of poultry flocks in these zones, there was a continued spread of the virus by mechanisms which are not clearly understood [8,10,22]. This spread only came to an end after the control measures had led to the culling of a large proportion of farms in the affected regions [8]. For the farmers, this meant incurring economic losses through and emotional burden of lost stock. Moreover, after a debate accruing from the 2001 Foot-and-Mouth Disease epidemic in the UK and the Netherlands, public opinion turned against the (large-scale) preventive killing of healthy animals; deeming it unethical [90,91]. Hence the Dutch government is seeking alternative control measures to (large-scale) preventive culling, with emergency vaccination being the preferred strategy. However, in comparison with preventive culling, emergency vaccination would have the important disadvantage that its effect suffers from a 7 to 14 days protection delay [92]. This delay would prolong the time until epidemic control is obtained especially in the high density poultry areas (de Jong and Hagenaars [40] and the references therein). Thus the identification, testing and implementation of supplementary control strategies such as improved biosecurity are required. Identification of such strategies requires us to better understand the neighbourhood transmission (i.e., the indirect spread of the virus to farms neighbouring an infectious farm) of the virus.

Plausible mechanisms include movements of humans (professional and non-professional visitors, employees and farmers themselves), vehicular traffic (for example, delivery trucks), other fomites (such as tools, cell phones and shared farm equipment) and other vectors such as wind, rodents and insects [22,25,49,93,94]. These transmission events involve transportation of the virus either in contaminated litter, faeces or skin and feathers that can colloid on the fomites or the vectors' body. Therefore, in order to better control neighbourhood transmission, we need to understand deeper the steps involved in the whole virus dissemination process; a quite complex task.

Following potentially infectious contacts i.e. exposures, the probability of HPAI virus transmission may be contact-specific but will also depend on the contact patterns: i.e., the frequency of contacts and the contact network [46,95,96]. This interplay illustrates the need to determine the probability of virus transmission by a given type of contact during an epidemic. A combination of the estimated probabilities and the information on contact patterns can then be used to rank the individual contact risks and to assess risks of spread between different densely populated poultry areas. The resulting ranking is also important to guide further research and biosecurity implementation.

During the Dutch HPAI epidemic in 2003, the National Inspection Service for Livestock and Meat (RVV), responsible for the implementation of animal disease legislation and eradication of outbreaks of OIE listed diseases, was tasked with collecting epidemiological data and tracing of upward and downward contacts to and from infected farms. Using this data, Thomas and co-workers [22] performed a risk factor analysis to establish the factors that may have been responsible for the

introduction of the virus on each of the farms involved. They found an increased risk of HPAI virus introduction in layer-finisher type poultry compared to other poultry types. Their analysis gave some clues on the risk factors for HPAI virus introduction such as poultry type and flock size. However, it is also important to gain insight into the transmission routes of the virus including the absolute risk of infection for given types of indirect contact between farms, an aspect addressed by the type of analysis we perform in this study. Since contact frequency and the per-contact probability of virus transmission partly determine the risk that a given category of contacts poses, the results of this analysis may facilitate a risk classification of these contacts. Such a classification is vital in the design of improved biosecurity and possibly other control strategies.

Our analysis aims to give quantitative insight into the role of the different between-farm contacts in the spread of the virus during an epidemic. We focus on the specific contacts that occurred during the HPAI (H7N7) epidemic in the Netherlands and estimate the probability of HPAI virus transmission attributable to each type of contact. Using published genetic data obtained by sequencing most of the samples collected during the epidemic [97], we assessed the consistency of our estimates with the genetic data. With these results, we provide scientific support to improve biosecurity measures to prevent transmission.

Materials and methods

Data

We used two sets of data collected during the Dutch 2003 HPAI epidemic. One of the datasets was collected via a standardized field epidemiology investigation form of the RVV [98]. It included detailed information about day-to-day visits to all farms (infected and non-infected) such as visits for deliveries of farm inputs and for off-transport of outputs as well as professional and non-professional visits. In compiling this particular data, a follow-up to the visits mentioned by the farmers was made where possible. The preliminary data were cross-checked in detail and completed by the tracing unit of the crisis centre using the files obtained from the poultry-related businesses involved. This dataset captured information on a total of 614 visits originating from 203 infectious farms. Out of these visits, 381 were to infected farms. The total number of receiving farms was 325 of which 149 were ultimately infected. The other dataset was entirely about the visits that occurred in relation to measures aimed at controlling the epidemic (crisis organisation contacts). These included visits for: screening (i.e., the clinical inspection of poultry in the surveillance zone), tracing (i.e., the follow-up of visits from infected farms), indexing (i.e., the valuation of the flocks to be culled), and culling activities by the RVV [93]. From this dataset we selected visits to a farm that occurred up to seven days prior to and excluding its day of suspicion. For these contacts, we only considered same-day visits i.e., those that occurred on the same day that the person had visited an infectious farm.

In both datasets we could also find HPAI-related details such as the status and dates of clinical suspicion and stamping out for both the infected source farm and

receiving farms. Since we could not identify a potentially infectious traced visit for all the ultimately infected farms, we introduced a category of 'unknown' contacts over different distance ranges. A farm was assigned one unknown contact per day when it was in the vicinity of an infectious farm. We chose three distance ranges (and hence three different unknown contact types) namely, 0-1 km, 1-3 km and 3-10 km of an infectious farm and assigned the unknown contacts accordingly. Details of these and all the other visits are given in Table 1.

Table 1. The description of the contacts extracted from the three datasets based on the assumed infectious and potential virus-introduction periods of this study

Type of contact	Description
Feed delivery contact	A truck delivers feed to an infectious farm and proceeds to a susceptible farm.
Egg transport contact	A truck picks eggs or trays from an infectious farm and proceeds to a susceptible farm.
Rendering contact	A routine pick up of dead animals (not related to culling) occurred on an infectious farm and proceeds to a susceptible farm.
Other-professional contact*	A person (for example; veterinarian, dealer, advisor, technicians, and 'unspecified-others') visits an infectious farm and proceeds to a susceptible farm.
Crisis organisation contact	Person-contact for epidemic control activities such as screening, tracing, indexing, and culling that visited an infectious farm and proceeded to a susceptible farm.
Unknown contact:0-1 km**	Contact assigned to farm for every day that it is within 1 km of an infectious farm.
Unknown contact:1-3 km**	Contact assigned to farm for every day that it is between 1 and 3 km of an infectious farm.
Unknown contact:3-10 km**	Contact assigned to farm for every day that it is between 3 and 10 km of an infectious farm.

*The variable is a combination of related traced variables.
**A farm was assigned one unknown contact per day that it was in the vicinity of an infectious farm within the indicated distance range.

For each farm (infected or not) in the dataset, we extracted (and tabulated) all its exposures. In the summary table for the analysis, we indicated, for each contacted farm, the type and number of exposures as well as its ultimate status. A farm was deemed exposed if the visit occurred during the period when the virus was likely to have been introduced onto the receiving farm, here referred to as the potential virus-introduction period. Due to the uncertainty about the actual day of virus introduction, both the potential virus-introduction and infectious periods were assumed to begin seven days prior to the day of clinical suspicion, corresponding to the estimated farm infectious periods during the epidemic (i.e., 7.3 and 6.9 days for the two regions affected) for the period after epidemic detection [8]. The potential virus-introduction period lasted until the day before clinical suspicion while the infectious period lasted up to seven days after stamping out. This extended infectiousness was based on the hypothesis that the stamping out did not immediately rid the entire farm and its surroundings of all infectious material.

Data analysis

If p_i is the probability of infection per type $-i$ exposure, then the cumulative probability of a farm escaping infection $\left(P_{\text{escape}}\right)$ following a series of exposures is $\prod_{\text{all i}}\left(1-p_i\right)^{C_i}$ where C_i is the total number of type $-i$ exposures and the compliment $(1-P_{\text{escape}})$ gives the probability of infection. In this case, we consider p_i to be the conditional probability of virus transmission per contact i.e., the probability that a given contact transmitted the virus given that the contact occurred and that it originated from an infectious farm.

To estimate these probabilities, we used a maximum-likelihood approach. The likelihood function was given by

$$L\left(p_i \mid C_{i,d}^{\text{inf}}, C_i^{\text{esc}}, w_d\right) =$$

$$\left(\prod_{\text{all infected farms}}\left(1-\prod_{\text{all i}}\left(1-p_i\right)^{\sum_d w_d C_{i,d}^{\text{Inf}}}\right)\times\left(\prod_{\text{all i}}\left(1-p_i\right)^{\sum_d (1-w_d) C_{i,d}^{\text{inf}}}\right)\right)\times\prod_{\text{all escaping farms}}\left(\prod_{\text{all i}}\left(1-p_i\right)^{C_i^{\text{esc}}}\right),$$

where i indexes the exposure-type, d indexes the day-number (days before clinical suspicion day) that the contact occurred, $C_{i,d}^{\text{inf}}$ is the number of type $-i$ exposures to a case farm occurring d days before clinical suspicion, C_i^{esc} is the total number of type $-i$ exposures to a non-case farm, w_d is the 'weighting factor' representing the probability that infection occurred through exposures occurring on day d (see below), $\left(1-\prod_{\text{all i}}\left(1-p_i\right)^{\sum_d w_d C_{i,d}^{\text{Inf}}}\right)$ is the probability of a farm being infected, $\left(\left(1-p_i\right)^{\sum_d (1-w_d) C_{i,d}^{\text{inf}}}\right)$ is the probability of a farm escaping infection by type $-i$ exposures, and $\left(\prod_{\text{all i}}\left(1-p_i\right)^{C_i^{\text{esc}}}\right)$ is the probability of a farm escaping infection throughout the epidemic.

In this analysis, we assumed that: 1) the 'exposure' period started seven days prior to and lasted until the eve of clinical suspicion, 2) the infectious period began seven days prior to the day of clinical suspicion and lasted up to seven days after stamping out, 3) the conditional probability of infection was fully dependent on the contacts indicated in Table 1, and 4) the per-contact probability of infection by the traced contacts is independent of the distance between the source and receiving farms.

We used Mathematica 8 (Wolfram Research, Inc.) to perform the maximisation procedure. The 95% Confidence Intervals (CI) for the maximum likelihood estimates were computed using the likelihood ratio test. We quantified the contribution of the different contacts to the epidemic in terms of the number of new infections that they may have caused. This was obtained by multiplying their estimated per-contact probability with their frequency.

As an introduction can only occur on one day, we can only allow for the uncertainty about when this day was by giving weights to each of the possible introduction days with these weights adding up to one. For the base model, we used a uniform distribution to obtain $w_d = \frac{1}{7}$. In other words, we assumed that each of the seven days of the probable period of virus introduction was equally likely to be the actual day of virus introduction. However, we also checked the outcomes based on different distributions in the sensitivity analysis.

Sensitivity and bias analyses

Sensitivity analysis: We performed a sensitivity analysis to ascertain the effect, on the probability estimates, of the possible uncertainty in defining the distribution underlying the actual day of virus introduction over the assumed period. We performed this analysis by re-running the calculation with different distributions underlying the estimation of the weighting factor w_d. We assessed two other distributions in which the estimated weighting factors w_d were adjusted to sum to one over the 7-day period, namely; 1) a distribution in which the probability is decreasing exponentially over the 7-day period at a rate determined by the survival of HPAI virus in manure (in this case 14 days [99]) and, 2) a unimodal distribution with the most likely day being 4 days prior to the day of clinical suspicion. In the second case, we used a discretized normal distribution with a truncated domain and $\sigma = 1$ day. In both cases the assumed distributions were normalised to sum to one.

Potential difference in tracing efforts on case and non-case farms: We hypothesized that, during the epidemic, the tracing process may have been more rigorous on case farms compared to the non-case farms. We explored the effect of this possibility by considering a scenario where an under-representation of the contacts to the escaping farms—for example due to a more lax attitude of the tracing teams when on non-case farms—could have occurred. We estimated the maximum effect that this would have on the estimated probabilities in the following 3 steps: 1) if we let P_{tr} be the tracing probability of a contact, this would be the exact probability of tracing a contact to a non-case farm if no back-tracing at all was made at the non-case farm, 2) with back-tracing in place for the case farms, the probability of tracing their contact would be

$1-\left(1-P_{tr}\right)^2$ and finally, 3) the maximum bias due to under-representation occurs at the worst tracing level and would be given by $\lim_{P_{tr}\to 0}\left(\frac{1-\left(1-P_{tr}\right)^2}{P_{tr}}\right)=2$.

Validation against genetic data

In order to validate the estimated per-contact probabilities, we used the genetic data obtained by sequencing the majority of the samples collected for outbreak farms during the epidemic [97]. In this way, we used the genetic data to validate the estimated probabilities per contact: too few or too many genetic matches would cast doubt on the estimated probabilities. The approach developed for this validation is described below and in the Supporting Information file Text S1.

With the contact inclusion criteria described under Data section, we extracted traced contact pairs, i.e. farm pairs (A, B) in which at least one contact originating from a then deemed infectious farm A to a hitherto susceptible (but ultimately infected) farm B, and occurring within the exposure period of farm B, was traced. We then used the genetic information generated from the majority of the samples taken from the affected farms during the epidemic as reported by Bataille et al. [97] in Figure S2 of their Supporting Information to identify which pairs had virus sequences for both farms. For those pairs (i.e., with complete genetic information), we compared their genetic sequences to ascertain which ones were sufficiently "matching" for transmission between A and B not to be unlikely. The number of genetically matching pairs, minus an estimate of the expected number of "by-chance" genetic matches, was then compared to the predicted number of pairs (amongst those with complete genetic information) in which virus transmission occurred ("transmission pairs") $\left(N_{\text{predicted}}\right)$. This number was estimated from the overall expected number by scaling it according to the expected contribution of the 28 contacts, relative to that of the 56, based on the estimated probabilities.

We considered four different (sets of) criteria for determining whether a contact farm pair (A, B) represents a genetic match. These (sets of) criteria differ in the level of genetic overlap required between the sequences from farm A and farm B to qualify as a genetic match. The most liberal criterion we considered was that all mutations in the virus of farm A compared to farm 1 (i.e., the first outbreak) were also found on farm B, i.e. when going from A to B no mutations are lost. This criterion is necessary because it is highly unlikely for the virus to lose mutations (i.e. undergo backward mutation) between source and receiving farms. In the other three, in addition to having no lost mutations, we permitted only a specific number/range of additional mutations: allowing no additional mutations at all, allowing ≤ 3 and, ≤ 6 additional mutations. For each criterion, we calculated an expected number of transmission pairs by subtracting an estimate of the number of 'chance matches' from the total number of genetic matches (for details see Supporting Information file Text S1).

Results

With our selection criteria applied to the first dataset i.e., the data from the epidemiological investigation by the RVV, we were able to extract at least one traced exposure for 36 (i.e. 15%) ultimately infected farms and the number increased to 44 (i.e. 18%) upon including the crisis organisation contacts. With the complete dataset (i.e., the latter two together with the extracted unknown contacts), 227 (i.e. 94%) ultimately infected farms had been exposed. Thus with all the available and modelled data, all but 14 infected farms had either a traced exposure or it was in the neighbourhood of an infectious farm (unknown contacts).

In Table 1, we present a description of both the potentially infectious contacts recorded during the HPAI (H7N7) epidemic in the Netherlands in 2003 and the unknown contacts extracted for purposes of this study. In Table 2, we present the extracted number of contacts that met our inclusion criteria and their mean estimates of the per-contact probability of virus transmission (and their accompanying 95% CI). We also present in the same table the percentage (and 95% CI) of infections potentially caused by these contacts and the results of the sensitivity analysis.

Table 2. The number of contacts, the estimated per-contact transmission probabilities (95% CI), and the percentage of infections caused for the potentially infectious contacts during the HPAI (H7N7) epidemic in the Netherlands in 2003

Contact type	*Total number of contacts (to a case farm)*	*Per-contact probability of infection (95% CI)*	*Percentage of infections caused (% of 227 cases)*	*Sensitivity analysis: w_d~exponential decay function*	*Sensitivity analysis: w_d~unimodal distribution*
Unknown contact:0-1 km	27700 (3048)	0.0000570 (0.00 - 0.00044)	0.70 (0.00 – 5.37)	0.0000449	0.0000586
Unknown contact: 1-3 km	190846 (25035)	0.000413 (0.00031 – 0.00052)	34.72 (26.06 – 43.72)	0.000414	0.000430
Unknown contact: 3-10 km	1466564 (171021)	0.0000895 (0.000076 – 0.00010)	57.82 (49.10 – 64.61)	0.0000906	0.0000913
Crisis organisation contact	272 (16)	0.00110 (0.00 – 0.012)	0.13 (0.00 – 1.44)	0.000	0.000
Feed delivery contact	144 (23)	0.0414 (0.0043 – 0.085)	2.63 (0.27 – 5.39)	0.0342	0.0261
Egg transport contact	15 (8)	0.308 (0.16 – 0.48)	2.04 (1.06 – 3.17)	0.305	0.303
Other-professional contact	16 (5)	0.133 (0.023 – 0.29)	0.94 (0.16 – 2.04)	0.130	0.000
Rendering contact	12 (4)	0.246 (0.10 – 0.43)	1.30 (0.53 – 2.27)	0.239	0.179

Apart from the unknown and crisis organisation contacts, feed deliveries had the lowest per-contact probability of virus transmission of 0.0414 and potentially caused 2.63% of the new case farms while the egg transports had the highest per-contact probability of 0.308 and may have potentially caused 2.04% of the new case farms. The probability of virus transmission per crisis organisation contact was estimated to be

0.0011 and these visits may have caused 0.13% of the new case farms. The majority (92.54%) of the new cases were caused by the unknown contacts within the distance bands of 1-3 km and 3-10 km.

Analysing the sensitivity of the estimated probabilities to the assumed distribution underlying the actual day of virus introduction over the 7-day period, the outcomes from using the two alternative distributions (i.e., one with an exponentially decreasing probability and the other with unimodal distribution) were compared with those of the default distribution (i.e., uniform distribution). The estimates were very similar for most of the exposure types. The only differences found, but these were small, were in the per-contact probabilities for the crisis organisation contacts for both alternative distributions and the other-professional contacts for only the unimodal distribution (see Table 2). For both alternative distributions, the probabilities per crisis organisation contact were within the 95% CI of the default distribution whereas for the unimodal distribution, the per other-professional contact probability reduced from 13.3% to 0.0%. This reduction is a consequence of the very low weights w_d assigned by the unimodal distribution to the days on which these contacts occurred. Three of the five contacts to ultimately infected farms occurred seven days prior to the day of clinical suspicion while the remaining two occurred four days and one day prior to the day of clinical suspicion.

With respect to the effect of the potential difference in tracing efforts on case and non-case farms— hence a possibility of under-representation of the contacts to non-case farms, we found that, with the worst tracing efforts, the contacts to case farms would be twice as likely to be traced as those to non-case farms. This implies that, at worst, the estimated probabilities could be double their 'unbiased' counterparts.

There were 56 traced contact pairs in which virus transmission may have occurred i.e. contacts from an infected farm to a newly infected farm. From the genetic data of the same outbreak [97], complete genetic information was available for 28 of these pairs (see Table S1 in the Supporting Information). Using the estimated per-contact transmission probabilities and the numbers of each contact-type, we estimated that 15.96 outbreaks were explained by the traced contacts (Table 2). After rescaling, we obtained the predicted number of transmission pairs with matching genetic information $N_{\text{predicted}}$ as 8.96. The lower and upper 95% confidence bounds of $N_{\text{predicted}}$ were estimated to be zero and 19 pairs respectively.

In Supporting Information Table S2, we present results of the pairwise genetic comparison of the 28 pairs for our different criteria of defining a genetic match. We observe (Table S2) that using the most strict criterion of requiring a 'perfect' genetic match between contact pairs (A, B) i.e., having no lost and no additional mutations when going from A to B, we estimated that virus transmission may have occurred in two pairs, reducing to 1.85 pairs upon subtracting the expected number of chance matches. If we defined a contact pair (A, B) to be a genetic match if there were no lost mutations when going from A to B and permitting any number of additional mutations, the number of transmission pairs was estimated to be nine, reducing to 7.23 pairs when adjusted

for chance matching. Restricting the number of allowed additional mutations to ≤ 6 or to ≤ 3 yields five matching pairs in both cases, reducing to 3.98 and 4.26 pairs respectively after subtracting the expected number of chance matches. All these results are within the 95% confidence bounds of the predicted number of transmission pairs with matching genetic information and hence the observed and predicted numbers are consistent.

Discussion

The mechanisms of HPAI virus spread between farms are poorly understood; it has been hypothesized that the indirect between-farm contacts play a role [22,25,49,93,94]. The frequency and the transmission effectiveness of these contacts determine their virus transmission rates. Here we perform a quantitative assessment of the contribution of indirect contacts to the spread of the virus between farms during the 2003 HPAI epidemic in the Netherlands. During this epidemic, potentially infectious contacts to both infected and escaping farms were traced. We use the collected data to quantify the per-contact probability of virus transmission between farms.

The estimated conditional probabilities of virus transmission are presented in Table 2. In terms of per-contact risk, the estimates reveal that egg transports have the highest risk with approximately 31% chance of transmission followed by the rendering visits with a chance of transmission of 25%. The unknown contacts in the distance band of 0-1 km have the lowest risk per contact although, as is clear from the 95% confidence bounds, its estimated per-contact probability is not significantly different from those of the other unknown contact categories. We expect that the implementation of preventive culling within 1 km of an infectious farm during the epidemic [8] has had a (strong) censoring effect on the detection of infected farms with 1 km of an infectious farm, thus producing a downward bias on the transmission probability per unknown contact within 1 km. We note that the estimated per-contact probability for the unknown contacts within the distance band of 1-3 km being higher than that of the 3-10 km distance band contacts reveals a distance-dependent transmission risk similar to the one found by Boender et al. [10].

Generally, most exposure-types (all except the crisis organisation contacts) made a substantial contribution to virus transmission during the epidemic. We note that the estimated per-contact probability of virus transmission by the crisis organisation contacts is 0.0011 and may have caused 0.13% of the infections. We note that when ignoring all other exposure types, i.e. considering the crisis organisation contacts alone in a separate analysis, we estimated a probability of 0.0327 per contact corresponding to 3.92% of the infections. This probability estimate is in agreement with the estimated maximum probability of virus transmission by a ‘control-person’ per visit of 0.037 reported by te Beest et al. [93] based also on a separate analysis of crisis organisation contacts only.

We hypothesize that the lower probability of infection per crisis organisation contact compared to that of the other-professional contacts which are almost of the same nature indicates that the epidemic control teams have better biosecurity than other visitors. The lower per-contact probability of infection per feed delivery compared to egg transport may be due to the difference in degree of contact and the re-use of egg trays. Unlike egg pick-up where the eggs have to be picked from the egg room, feed delivery may not involve accessing storage rooms or poultry houses. In most cases, the feed truck's delivery tube is directly connected to the feed storage from the outside thereby reducing the risk of farm contamination.

In the sensitivity analysis, we find that the majority of the estimates are robust to the assumed distribution of the most likely day (among the seven days) of virus introduction. For the few sensitive (but less contributing) contact types, we concentrate on the results obtained using the uniform distribution as this assumes the least prior knowledge on the actual moment of disease introduction on the farm. Regarding the effect of a possible difference in tracing efforts on case and non-case farms, we have argued that an under-representation of the contacts to non-case farms may have at most doubled our probability estimates i.e., compared to the 'ideal' situation where the tracing efforts are the same for the case and non-case farms.

The pairwise comparison of the genetic information of the contact pairs (Tables S1 and S2 in the Supporting Information) shows that the very low numbers of new infections explained by the traced contacts in our analysis is consistent with the genetic data. This genetic data has been used to construct transmission trees in reference [97] and in more detail in reference [100]. Our present analysis focused on estimating per contact transmission probabilities for the different between farm contact types using the contact tracing data. Note that there is no straight forward way to directly include genetic data in an estimation of the per contact transmission probabilities as the sequencing data only gives information on the case farms and not on the contact farms that escaped infection. However, both data types (i.e., genetic and epidemiological) can be combined within the same analysis to, for example, determine transmission pathways. This approach was proposed by Cottam et al. [101] in their analysis of part of the 2001 FMD epidemic in UK.

Stegeman and co-workers [46] performed a similar analysis on the 1997/1998 Classical Swine Fever (CSF) epidemic in the Netherlands. The common contact types in both studies are the 'person' (similar to 'other-professional') and rendering contacts. Perhaps remarkably, the estimated transmission probabilities for these contacts in our HPAI study are respectively two and four orders of magnitude higher than those estimated in the CSF study. These differences are mainly due to a difference in total numbers of between-farm contacts, with 16 and 12 for the HPAI epidemic (affecting 255 flocks) compared to 2468 and 10102 for the CSF epidemic (affecting 429 farms), respectively. The much higher numbers of contacts in the CSF epidemic are explained in part by the much longer duration of the epidemic: 15 months in comparison to the 3 months that the HPAI epidemic lasted. The difference in number of contacts is likely to be also related in part to the fact that the CSF epidemic was more spatially extended

compared to the HPAI epidemic. As a result, there were more new outbreaks occurring outside existing stand-still areas (in which onward contacts are more restricted) for the CSF epidemic as compared to the HPAI epidemic.

With our contact inclusion criteria, 44 infected farms have at least one traced exposure i.e., excluding the 'unknown' contacts. The outbreaks that could not be linked to any known potentially infectious contact may not only be attributed to the inability to trace all targeted contacts. Rather, they may serve as a hint about the presence of other (un-targeted and hence untraced or even untraceable) mechanisms. This highlights the need to better understand the possible mechanisms of untraced transmission.

It is important to realize that the probabilities estimated are conditional on the contact originating from an infectious farm and do not represent the actual risk of HPAI virus transmission by these contacts during the epidemic. We also emphasize that care should be taken when interpreting the per-contact probability estimate for the rendering contacts due to the possible correlation between this contact-type and the increased mortality which could have occurred during the silent spread period of the virus on the farm i.e., the virus could have already been circulating undetected on the receiving farms. Nevertheless, the probability estimates together with the risk-based ranking for the different contacts obtained in this study can help design better control strategies against HPAI virus transmission between-farms by these contacts.

All in all, after estimating the per-contact probability of virus transmission for the different contacts, we conclude that all the identified contacts made a substantial contribution to the risk of virus transmission between farms. Therefore, any measures to reduce on their frequency and to improve biosecurity during all these contacts are potentially worthwhile. The fact that the 'unknown' contacts contributed the most (causing 93.24% of the infections among themselves) emphasizes the need for a better understanding of the mechanisms underlying virus transmission.

The findings of this study contribute to the greatly desired understanding of the mechanisms of indirect transmission of HPAI virus between farms. Our results suggest that, apart from the unknown contacts, egg delivery contacts are interesting targets for improvements in biosecurity due to their high per-contact probability (31%) in infecting the receiving farms. They further suggest that the biosecurity applied to the crisis organisation contacts seems to be adequate at least for preventing the persons themselves from becoming important fomites between registered visits. Overall, these findings provide a scientific basis to conduct further studies, epidemiological or otherwise, to evaluate the impact of improved biosecurity and minimized contact-frequency in controlling the between-farm spread of HPAI virus during epidemics. The knowledge gained in this study can further be supplemented by research aimed at disentangling the ambiguous category of 'unknown' contacts defined in this study.

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Supporting Information

Further details on the validation against genetic data

Number of chance matches

The number of chance matches was estimated as follows: from Figure S2 of Bataille et al.[97], we counted the total number of possible outbreak farm pairs that met a specific criterion for defining a genetic match. We then divided the outcome by the total number of possible outbreak farm pairs to obtain the probability of having a matching pair just by chance. By multiplying this probability with the number of contact pairs with complete genetic information, we obtained the expected number of chance-matches.

Confidence bounds for the predicted number of genetic matches

The 95% confidence bounds of $N_{\text{predicted}}$ were calculated based on a 'mean' per-contact probability $\left(P_{\text{combined}}\right)$ and its lower $\left(P^{L}_{\text{combined}}\right)$ and upper $\left(P^{U}_{\text{combined}}\right)$ 95% confidence bounds by grouping all the traced contacts into one category and re-running the analysis described under Data analysis section in main text. Then the estimated P^{L}_{combined} and P^{U}_{combined}, after multiplication by the probability of having a pair with complete genetic information, were each used as probabilities of a binomial distribution for the number of observed genetic matches, with the total number of traced contact as the binomial total. The 2.5 percentile of the binomial distribution corresponding to P^{L}_{combined} and 97.5 percentile of that corresponding to P^{U}_{combined} gave the 95% confidence bounds of $N_{\text{predicted}}$.

Table S1. Summary of genetic differences between isolates from the source and receiving farms for the traced contact pairs

Contact pairs (source, receiver)	Exposure type	Additional mutations[$]	Lost mutations [$]
(001,007)	Rendering visit		**
(001,010)	Rendering visit	3	0
(001,022)	Rendering visit	3	0
(013,079)	Rendering visit		*
(003,098)	Egg transport		***
(010,080)	Egg transport	4	3
(018,098)	Egg transport		**
(046,052)	Egg transport	2	1

(046,061)	Egg transport	0	0
(027,036)	Egg transport	7	1
(034,033)	Egg transport		*
(027,082)	Egg transport	8	1
(022,034)	Feed delivery		**
(091,129)	Feed delivery	9	0
(117,081)	Feed delivery		**
(076,114)	Feed delivery		**
(090,161)	Feed delivery	1	2
(091,140)	Feed delivery	11	0
(106,127)	Feed delivery		***
(113,166)	Feed delivery	1	0
(030,061)	Feed delivery	2	5
(091,145)	Feed delivery	10	0
(113,180)	Feed delivery	12	5
(013,125)	Feed delivery		*
(016,053)	Feed delivery		**
(214,234)	Feed delivery		Deletion
(093,177)	Feed delivery		*
(221,232)	Feed delivery		Deletion
(090,122)	Feed delivery		**
(043,106)	Feed delivery		**
(124,156)	Feed delivery		*
(094,090)	Feed delivery	8	4
(124,189)	Feed delivery		***
(106,149)	Feed delivery		***
(106,149)	Feed delivery		***
(003,030)	Other-professional visit		*
(016,030)	Other-professional visit	5	2
(005,043)	Other-professional visit	0	0
(155,154)	Other-professional visit	2	1
(133,200)	Other-professional visit		***
(051,073)	Crisis organisation contact		**
(108,073)	Crisis organisation contact		**
(023,033)	Crisis organisation contact	8	1
(001,033)	Crisis organisation contact	10	0
(131, 171)	Crisis organisation contact	6	11
(128, 171)	Crisis organisation contact		*
(097, 083)	Crisis organisation contact		***
(117, 097)	Crisis organisation contact		**
(117, 097)	Crisis organisation contact		**
(051, 108)	Crisis organisation contact	5	3
(053,108)	Crisis organisation contact		*

(054,108)	Crisis organisation contact	10	5
(149,169)	Crisis organisation contact		*
(107,110)	Crisis organisation contact	4	3
(105,123)	Crisis organisation contact	11	6
(102,123)	Crisis organisation contact	3	2

* sample from source farm was not yet sequenced
** sample from receiving farm was not yet sequenced
*** samples from both receiving and source farms were not yet sequenced
$ Comparison is between the virus on recipient farm and that on the sender farm using virus on farm 001 as the root, i.e. additional mutations are the number of mutations that the sender farm's virus is closer to the root than the recipient farm's virus. In addition the recipient farm's virus may differ from the sender farm's virus because of mutations that the sender farm's virus had and the recipient farm's virus has lost. If the number of mutations lost is equal to zero, the sender farm and the recipient farm are on the same branch with the sender being closer to the root. We assume that when the virus on the sender farm has a deletion the virus on the recipient farm cannot be without that deletion so this combination cannot be a transmission event. The mutations are counted based on Figure S2 of Bataille et al. (2011).

Table S2. The observed number of genetically matching pairs (A, B), within 28 pairs of outbreak farms linked by traced contacts, for different criteria of defining a genetic match. Our analysis of transmission probabilities of contact types predicts transmission to occur from A to B for an expected number $N_{\text{predicted}}$ of 8.961 pairs within the 28 (due to the traced contacts). This number is obtained by multiplying the expected number of transmission pairs (which is 15.96) by a scaling factor of 0.5615. This scaling factor is the expected contribution of 28 contacts relative to that of the 56 based on a weighted count of the contacts of each type (given in Table S1), using the per-contact transmission probabilities as weights.

	No lost mutations in B compared to A	No lost mutations AND ≤6 additional mutations in B compared to A	No lost mutations AND ≤3 addition mutations in B compared to A	No lost mutations AND no additional mutations in B compared to A
#matching pairs (out of the 28 pairs); M	9	5	5	2
Counts of pairs meeting criterion; n_c	2125	1231	886	182
'probability of chance agreement'; p_c=n_c/(184x183)[$]	0.06311	0.0366	0.0263	0.00541
Expected number of chance matches; R=p_c*28	1.77	1.02	0.74	0.15
Matching pairs corrected for chance (M-R)	7.23	3.98	4.26	1.85

[$] The total number of pairs possible is 184×183 as there are 184 outbreak farms for which sequencing information is available and for any two farms A and B, the pair (A, B) is different from (B, A).

Chapter 4

Avian Influenza transmission risks: analysis of biosecurity measures and contact structure in Dutch poultry farming

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Abstract

In the 2003 epidemic of highly pathogenic avian influenza in Dutch poultry, between-farm virus transmission continued for considerable time despite control measures. Gaining more insight into the mechanisms of this spread is necessary for the possible development of better control strategies. We carried out an in-depth interview study aiming to systematically explore all the poultry production activities to identify the activities that could potentially be related to virus introduction and transmission. One of the between-farm contact risks that were identified is the movement of birds between farms during thinning with violations of on-farm biosecurity protocols. In addition, several other risky management practices, risky visitor behaviours and biosecurity breaches were identified. They include human and fomite contacts that occurred without observing biosecurity protocols, poor waste management practices, presence of other animal species on poultry farms, and poor biosecurity against risks from farm neighbourhood activities. Among the detailed practices identified, taking cell phones and jewellery into poultry houses, not observing shower-in protocols and the exchange of unclean farm equipment were common. Also, sometimes certain protocols or biosecurity facilities were lacking. We also asked the interviewed farmers about their perception of transmission risks and found that they had divergent opinions about the visitor- and neighbourhood- associated risks. We performed a qualitative assessment of contact risks (as transmission pathways) based on contact type, corresponding biosecurity practices, and contact frequency. This assessment suggests that the most risky contact types are bird movements during thinning and restocking, most human movements accessing poultry houses and proximity to other poultry farms. The overall risk posed by persons and equipment accessing storage rooms and the premises-only contacts was considered to be medium. Most of the exposure risks are considered to be similar for layer and broiler farms. Our results, including those on farmer opinions, are relevant for the communication with farmers and poultry-related businesses about practices and risks. We conclude by providing recommendations for improvement of control strategies.

Keywords: biosecurity; contact and neighbourhood structure; avian influenza; transmission pathways; risk assessment; poultry

Introduction

The poultry industry makes a significant contribution to the Dutch national economy. For example, in 2011, the average broiler population was more than 45 million birds, the laying hen population was close to 33 million birds and close to 900,000 tonnes of poultry meat and close to 10 billion eggs and egg products were exported [102]. The profitability of this industry was severely affected by the occurrence in 2003 of an H7N7 Highly Pathogenic Avian Influenza (HPAI) virus epidemic. In addition, this epidemic presented a risk to human health, both through transmission of the circulating virus to humans and through its assumed potential to seed the development of a new pandemic influenza strain [7]. The epidemic comprised 255 outbreak farms, 30 million birds were culled [8] and 89 people were infected, one of whom died [7]. The direct costs as a

result of bird deaths and depopulation amounted to €250 million, while indirect costs due to the epidemic were much higher [11,12].

Although, after diagnosis of the first cases of the epidemic, movement bans and other control measures were put in place, a continued spread of the virus was observed. In spite of the culling of contiguous flocks i.e., flocks that were in the neighbourhood of the outbreaks or that had had contact with an infected farm, this spread continued for weeks in particular in the high poultry density areas [8]. The transmission pattern during the epidemic indicates the presence of (untraced) indirect transmission routes or mechanisms that are not controlled by the European Commission's strategies. Hence in order to possibly improve control strategies, a better understanding of indirect transmission mechanisms is needed.

AI viruses may be introduced into poultry from reservoirs such as aquatic wild birds [2,4,5,26] but the mechanisms of their subsequent spread are partially unclear. Transmission of the virus through movements of humans (visitors, servicemen and farm personnel), vectors (wild birds, rodents, insects), air- (and dust-) related routes and other fomites (e.g., delivery trucks, visitors' clothes and farm equipment) have all been hypothesized [5,24,94,103-105].

It is therefore hypothesized that the risk of introducing the virus to a farm is determined by the farm's neighbourhood characteristics, contact structure and its biosecurity practices. On the one hand, neighbourhood characteristics include factors such as the presence of water bodies (accessed by wild birds), the density of poultry farms (together with the number and type of birds on these farms) and poultry-related businesses and the road network. The use of manure in the farm's vicinity is also deemed to be risky [2,22,25,26]. On the other hand, contact structure risk factors include the nature and frequency of farm visits. Therefore, a detailed analysis of the contact structure, including neighbourhood risks, and biosecurity practices across different types of poultry farms and poultry-related businesses could help the improvement of intervention strategies, biosecurity protocols and adherence to these, as well as contact tracing protocols. Farmers' perception of visitor- and neighbourhood- associated risks of virus spread is also important due to its relevance to adherence with biosecurity protocols, to contact tracing and to communicating advice to them.

The between-farm virus transmission risks may be split into two categories namely, introduction and onward-spread risks. The former entail the target farm's exposure through incoming contacts (human and fomite), through inputs such as feed and egg trays and through neighbourhood-related risks such as air-borne contamination. The latter can be through farm outputs (waste and non-waste), outgoing contacts (human and fomite) and contamination of the neighbourhood (e.g., through emissions from the farm). Therefore, we systematically analysed all day-to-day farm activities involving people and/or materials and/or equipment going in or out of the farm.

Through questionnaire-guided in-depth interviews, we sought information directly from the farmers and the poultry-related businesses. These interviews were aimed at gathering first-hand information about all the visits and processes involved and the accompanying biosecurity practices throughout the production round and across all poultry husbandry types. Other aspects of interest were the details about the farm's neighbourhood which are important in relation to indirect transmission risks. In the interviews, we aimed to learn more about possible risks in practice corresponding to the indirect contact types that are commonly hypothesized and/or that can be found in the tracing reports of the H7N7 epidemic in 2003 and any further possible indirect contact

types, in particular those that could provide a pathway for the untraced outbreaks (or 'neighbourhood infections').

Based on the gathered information, we generated a list of contact types that could serve as Avian Influenza (AI) transmission pathways. For these contact types, we then performed a qualitative risk assessment based on contact type, their corresponding biosecurity practices and contact frequency to ascertain which mechanisms are the most important to target during prevention and control.

Materials and methods

Study population

A cross-sectional study was performed with the aim of obtaining information on the types and frequency of the various day-to-day farm contacts and activities that can guide the determination of potential pathways of AI spread between poultry farms. The study involved 42 farmers and 18 poultry-related business representatives distributed all over the Netherlands. The stratum-specific sample sizes for the farms/firms to be interviewed were determined based on the underlying goal of making sure that all relevant types in the poultry chains were included. By sampling more farms from those strata representing a higher population proportion an attempt was being made to capture any between-farm variation in biosecurity practices present.

In 2009, there were approximately 687 broiler, 1097 layer, 248 breeder, 54 turkey and 66 duck farms and the layer farms comprised of approximately10% organic, 17% free range, 53 % deep litter and 20% cage farms [102]. From the national list available in the poultry production chain information (KIP) database, a random selection of 13 layer, nine broiler, four turkey, two duck, four broiler-breeder, eight pullet, one vaccine-egg producing and one organic/biological layer was made. For the poultry-related businesses, four hatcheries, two slaughterhouses, two egg grading companies, two feed mills, two manure plants/traders, two catching companies, two repair companies and two poultry veterinarians were included in the study.

Questionnaire design and data collection

Two questionnaires (one posed to the poultry farmers and the other to representatives of poultry-related businesses, both available upon request) were developed together with two (retired) experts who had worked in the Dutch poultry industry. They contained 125 and 296 closed, semi-closed and open type questions for the first and second round of interviews respectively. Participation in the survey was voluntary and for those that agreed to take part, appointments for the interviews to be held on the firm premises were made. All the selected farms and poultry-related businesses were visited and personal interviews conducted (in Dutch) between May and December 2009. In the first round of interviews, the questionnaire for the poultry farms was pre-tested on two farms, adjusted, and administered to the 42 farms. In the second round, the second questionnaire specifically designed for poultry-related businesses was administered to the 18 company representatives and professionals.

In addition to this data, we also needed a detailed list of locations of the various poultry-related businesses in the Netherlands for the assessment of the interviewed farms' neighbourhoods. Such a list could not be obtained from a single source; we generated it by extracting company information using 'Google' and combining the

results with information available on a Dutch website, the 'Pluimvee Gids' (Poultry Directory). Numbers of farms in the neighbourhoods were obtained from poultry farm location data.

Data management and descriptive analysis

Data gathered from the interviews were entered into a database file. Both data (from the interviews and the 'Google' extracted) were descriptively analysed to check the presence or absence of and/or determine the frequency of occurrence of events and practices that can promote virus transmission. In order to eliminate biased conclusions resulting from inaccurate reporting, farmer responses were compared with those of the poultry-related business representatives or were cross-checked with the poultry production chain information (KIP) database, maintained by the Product board for Poultry and Eggs (PPE), which contains the location of all commercial poultry farms in the Netherlands. Estimates of the number of farms and poultry-related businesses within a 5 km radius around each of the interviewed farms as provided by the farmer were compared to actual numbers obtained using farm location data and Geographic Information System (GIS). These findings were necessary for assessing neighbourhood-related contamination risks whereby we used the extracted numbers to infer qualitatively about the risk of farm contamination. It is also important in assessing the farmers' knowledge of their neighbourhood in terms of poultry density and its potentially associated risks.

Categorizing contacts and generating transmission pathways

The outcomes of the descriptive analysis were used to inform the generation of transmission pathways. We hypothesized potential pathways of virus transmission comprising of one or combinations of several of the reported activities. A pathway is here defined as a combination of activities and behaviours that can promote virus dissemination. Examples of pathways are: a person moving between farms without adhering to the farms' biosecurity protocols or a scenario in which poultry manure is used in the neighbourhood of a non-protected poultry farm.

We grouped the pathways into the following five categories: 1) between-farm movement of poultry, 2) between-farm movement of persons and equipment that access poultry houses, 3) between-farm movement of persons and equipment that access storage rooms only, 4) between-farm movement of persons and equipment that were only on the premises and, 5) neighbourhood-related contamination risks. The distinction of the four different between-farm movement categories was based on decreasing proximity of approach to the poultry by the persons or equipment when on the farm.

Qualitative risk assessment

This analysis used a risk ranking scheme based on the five pathways (Section 2.4) and the annual frequencies of contacts. Here (in contrast to other analyses), we concentrated on the end-of-chain broiler (hereafter referred to as broiler) and layer husbandry types since these two together present the majority of farms in the Dutch poultry industry. Our risk ranking scheme (Table 1) ranks the identified contacts in terms of the overall risk they pose, based on the combination of the per-contact risk

and the annual contact frequency. The highest per-contact risk level was assigned to category I pathways with the other categories being assigned systematically decreasing levels, based on decreasing proximity of approach to the poultry by the persons or equipment when on the farm. The frequency-related risk levels were assigned on an interval basis with more than ten contacts per year having the highest risk and the lowest risk interval being that of no contacts at all. The framework was applied to all the different contact types identified in layer and broiler farms to rank these contact types according to the risks posed, with the risk level in principle being dependent on the husbandry type.

Table 1. Proposed exposure risk classification scheme based on contact frequency, biosecurity practices and risk category

	Average number of contacts per year				
	≥10	< 10 and ≥3	< 3 and ≥ 1	< 1 and >0	≈0
Category I: movement of poultry between farms	n.o.*	Very high	n.o.*	Medium	Negligible
Category II: contacts accessing poultry houses	Very high	Very high	High	Medium	Negligible
Category III: contacts accessing storage rooms	Very high	High	Medium	Low	Negligible
Category IV: premises-only contacts	Very high	High	Medium	Low	n.o.*
Category V: neighbourhood risks **	Very high	n.o.*	Medium	n.o.*	n.o.*

**no contacts per year estimated, the risk is derived based on the number of farms or poultry-related businesses in the 5x5 km square neighbourhood.
*n.o. stands for 'not observed'.

Results

General interview findings

Only about 10% of the contacted farmers and firm representatives declined to take part and were replaced by other farmers or firm representatives within the same stratum. Both the study population and the geographical coverage of the selected enterprises was representative of the majority of the poultry husbandry types and the

regions in the Netherlands. From the farm neighbourhood analysis, most farmers somewhat underestimated numbers of poultry farms present within a 5 km radius around their farm. The average percentage by which their estimate underscored the GIS-extracted number was 51%.

Hired labourers are known to play a big role in inter-connecting farms. Here we found that 32 farms hired external labour of which seven accessed other poultry on the same day. However, they were not the only 'connectors' as some (twelve) farmers also reported themselves helping on other poultry farms. Furthermore, 27 farms had family members visiting poultry or poultry-related businesses of which nine entered poultry houses during those visits. The other enhancing factor of farm interconnections was the reported ownership of multiple locations for ten of the interviewed farms and the reported on-premises sale of farm products on one pullet and eight layer farms. Also worth mentioning is the practice of a multiple age system reported on eight of the interviewed farms as this may increase the risk of infecting remaining birds when off-premises poultry movements occur.

On 32 of the interviewed farms, the presence of other animal (non-poultry) species on the premises was reported. Manure use on agricultural fields in the neighbourhood of the farm was reported on ten of the interviewed farms. In terms of risk perception in relation to AI introduction, only 17 of the interviewed farmers perceived the presence of water bodies in their neighbourhood as posing a high risk and the farmers had divergent opinions about visitor-related risks (Figure 1). Farm visits were frequent - for example, feed mill technicians and veterinarians each accessed poultry houses and storage rooms on broiler farms for an average of 24.1 and 27 times per year respectively. More general results are presented in Table 2.

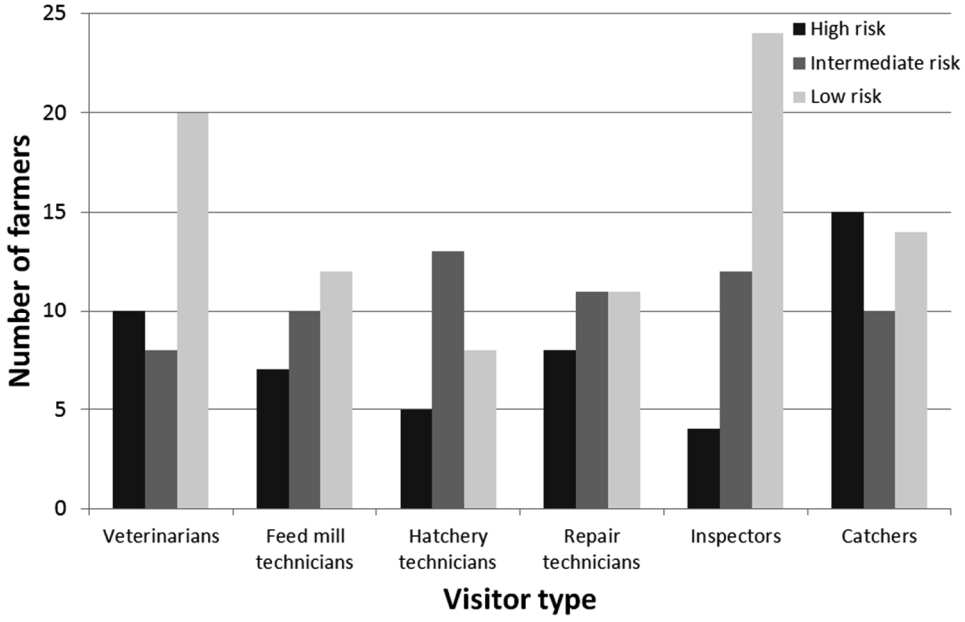


Figure 1. Number of farmers with a similar perception of the risk of Avian Influenza virus transmission associated with individual farm visitors.

Table 2. Summary of selected general information obtained from the questionnaire survey

	Layer	Broiler	Duck	Turkey	Pullet	Broiler breeder	Organic
Average number of birds on each farm (min, max)	41975 (3350, 130000)	52791 (180, 160000)	27500 (15000, 40000)	14724 (9000, 19000)	62844 (10000, 252900)	16250 (6000, 28000)	12000 (n=1)
Number of farms with >1 locations	5	1	0	3	1	0	0
Number of farms with outdoor run	4	0	0	0	1	0	1
Number of farms with multiple age system	4	0	1	3	0	0	0
Number of farms where the farmer visits other poultry farms	7	3	0	1	0	1	0
Number of farms where family members have access to other poultry	11	3	1	3	6	2	1
Average number of farms in 5 x 5 km: reported (extracted using GIS)	11 (37)	6 (20)	41 (113)	39 (64)	7 (30)	18 (19)	22 (33)
Number of farms where the farmer perceives the risk (in relation to AI introduction) posed by water bodies in the farm neighbourhood as high	5	5	1	1	2	3	0

Category I pathways: movements of poultry between farms

The already known movements in this category are during restocking and spiking (i.e., adding males in a flock) a destination farm. We also found that, on some of the farms, thinning by moving birds to other farms, to slaughterhouses, and to other poultry houses (on the same farm) is practiced. Thus this is an additional scenario of birds being moved from one farm to another.

The scenario of movement of infected day-old chicks means that these chicks are infected either at the hatchery or during transport. The risks for hatcheries to become contaminated with the virus are associated with a number of practices reported. These include the inconsistently applied biosecurity and/or the non-existent biosecurity facilities at some of the hatcheries as well as their non-adherence to farm biosecurity protocols.

Bringing personal items into hatchery production rooms may also be risky, as is the reported interchange of chick and hatch-egg delivery trucks coupled with the reported non-thorough cleaning and disinfection between trips. Re-use of setter trays on three of the interviewed hatcheries poses a threat of infection propagation inside the hatchery in case of non-thorough cleaning and disinfection. Also, between-hatchery business connections may be important determinants of the contamination risk. These connections occur through the sale of hatching eggs and chicks, and the associated contamination risk may also be enhanced by the reported sourcing of eggs from mixed sources.

Category II pathways: movements of persons and/or equipment between farms that access poultry houses

The already known (and confirmed by this study) contacts accessing poultry houses include professionals (and professional equipment, for example bird-catching and vaccination equipment) and non-professional visits (and equipment) by farm staff (temporal and permanent) or non-staff visitors. Some farmers themselves, their family members and the hired personnel accessed poultry on other farms and, on pullet farms, future flock-owner (purchaser) visits were also mentioned.

Among the reported, we identified biosecurity practices that contribute to these risks. These include the non-adherence to protocols and absence of farm biosecurity facilities and incomplete protocols (Table 3). There was inconsistent adherence to farm biosecurity protocols during restocking with six of the interviewed farms mentioning violations. In addition, most farms had showers that were never used and visitors did not always go through biosecurity transit rooms. Also, most of the interviewed farms lacked designated clean/dirty routes and only one interviewed farm had a marked walking route. Furthermore, all farms allowed personal belongings such as cell phones and jewellery into poultry houses and some farms shared equipment that was not always cleaned.

Violation of on-farm biosecurity protocols by the poultry-related company personnel renders their visits risky. There are several other visitor-type specific risky factors. These include veterinarians visiting up to 100 farms of different types per year and owning all the equipment they use. As reported, this equipment is not always thoroughly cleaned and disinfected. For the repairmen, visiting families with poultry on

non-official duties and working on farms with all types of poultry may increase their chances of contaminating the farms they visit.

The catching companies catch on all types of farms including day-old chicks at the hatcheries and hence pose a risk to the farms they visit. The crews of both catching companies used their own clothing and boots and the crew from one of them took catching cages, compressor, fork truck and dust covers on their work visits. Catching company representatives concurred with the farmers on the poor hygiene level of these items; both catching companies mentioned the visually unclean bird crates that they used on different farms.

Category III pathways: movements of persons and/or equipment between farms that only access storage rooms

The already known human and fomite contacts (and confirmed by this study) include feed mill and egg company staff contacts as well as repairmen and the equipment they use. Also, the between-farm use of trays, pallets, interfaces, and bird crates increases the risk of farm contamination. Most of the biosecurity practices that may contribute to these risks are the same as those of the category II pathways listed under Section 3.3.

The other notable factors found include the lack of or non-adherence to biosecurity protocols at egg packing stations and feed mills, the non-thorough cleaning and disinfection of the equipment, and multiple farm contacts through the multiple deliveries per day and/or trip. These companies expand the farm's network i.e., the number of farms that are connected or linked to each other. An example (from this study) of such a network expansion is the scenario in which egg packing companies obtained eggs from 100 and 150 farms with trays being exchanged between these farms and their clients getting eggs from other companies on the same day.

Table 3. Summary on selected farm biosecurity measures and visitor adherence to the available protocols for the different husbandry types

	Layers	Broilers	Duck	Turkey	Pullet	Broiler breeder	Organic
Number of farms (divided by total number reporting a particular visit) on which they do not use biosecurity transit rooms:							
Veterinarians	0/11	0/9	2/2	0/4	0/8	1/3	0/1
Feed mill technicians	1/12	1/8	2/2	0/2	0/2	1/2	0/1
Hatchery technicians	1/11	0/4	1/1	0/1	1/7	1/1	0/1
Repair technicians	1/10	0/5	2/2	0/4	0/3	0/3	0/1
Inspectors	3/13	3/9	2/2	0/4	4/8	3/3	0/1
Catchers	2/13	1/9	1/1	0/4	2/8	1/3	0/1
Vermin Control	1/5	0/1	n.o.*	n.o.*	1/3	n.o.*	0/1
Number of farms (divided by total number reporting a particular visit) on which they deviate from protocols:							
Veterinarians	1/11	0/9	0/2	0/4	0/8	0/3	1/1
Feed mill technicians	1/12	0/8	0/2	0/2	0/2	0/2	1/1
Hatchery technicians	0/11	0/4	0/1	0/1	0/7	0/1	0/1
Repair technicians	3/10	0/5	0/2	0/4	1/3	1/3	0/1
Inspectors	2/13	0/9	0/2	0/4	0/8	0/3	1/1
Catchers	2/13	0/9	0/1	0/4	0/8	2/3	1/1
Vermin Control	0/5	0/1	n.o.*	n.o.*	0/3	n.o.*	0/1
Number of farms (divided by total number reporting a particular visit) on which they enter poultry houses with personal items:							
Veterinarians	10/11	6/9	0/2	4/4	5/8	2/3	1/1
Feed mill technicians	10/12	5/8	0/2	2/2	2/2	0/2	1/1
Hatchery technicians	9/11	1/4	0/1	1/1	4/7	0/1	1/1
Repair technicians	10/10	3/5	0/2	3/4	2/3	3/3	1/1
Inspectors	4/13	1/9	0/2	1/4	1/8	0/3	1/1
Catchers	12/13	5/9	0/1	3/4	5/8	3/3	1/1
Vermin Control	4/5	1/1	n.o.*	n.o.*	1/3	n.o.*	1/1
Number of farms with/where:							
designated clean and dirty routes	1	0	0	1	1	0	0
hygiene protocols are violated during restocking	2	1	1	0	0	0	1
manure container and/or truck not always cleaned	2	3	0	0	3	1	0
measures to prevent contamination by manure	1	1	1	0	1	0	0

*n.o. stands for 'not observed'

Category IV pathways: movements of persons and equipment that only access the premises

These contacts include human contacts through input and output deliveries (e.g., feed and bedding) as well as manure pick-ups and having social gatherings of farmers on farm premises. The premises-only human contacts may also occur through the mentioned sale of eggs on the premises (or even in the poultry houses) and/or through delivery services of dead birds to the Central Veterinary Institute (CVI) for further investigation. We also found that only a few of the farms had biosecurity protocols for truck drivers with many arguing that there was no need since the drivers remained in the truck during most visits.

Premises-only contacts also include fomite contacts through shared farm equipment. These fomites may include the filling tube and the dust bags used during feed delivery, egg trays, pallets and manure containers. Also, the practice of allowing visitors to park on the premises without separate parking increases the chances of premises contamination as does the reported presence of other non-poultry species on the premises.

The poultry-related company practices that render these contacts risky are the delivery trucks lacking or not using the wheel disinfection systems that make multiple deliveries. Another risky practice is the random distributions of empty manure containers and egg trays. In addition, the practice of allowing trucks from different farms on the same company parking area at a given time may increase the risk of truck contamination before their subsequent visits. The already mentioned risk of expanding the farm's network (Section 3.4) also occurs through the extended sourcing of manure from up to 850 farms by the manure companies.

Category V pathways: neighbourhood risks

This category entails risks of indirect transmission attributable to the nature and frequency of poultry-related activities in the farm's neighbourhood. Factors such as the farm's proximity to other poultry farms and water bodies accessed by wild birds have been suggested to facilitate virus transmission. We add to this list the farm's proximity to poultry-related businesses and roads leading to these businesses due to exposure through windborne dispersal.

More to that, the presence of uncovered manure storages and the use of manure on agricultural fields in the neighbourhood of some of the interviewed farms further increase their contamination chances. These factors are facilitated by the lack of protection against contamination by manure on all except four farms, one of which used a six-meter wide strip with trees around the premises as a barrier. The resulting 'neighbourhood risks' may be facilitated by movements between the field and the farm by other animals, rodents, insects, wild birds, humans (in cases where contaminated dust colloids on their clothing and/or equipment), vehicular traffic as well as wind dispersal.

We identified on-farm biosecurity and other practices that may enhance these risks. These include poor farm waste management (for example, disposing of untreated waste water on the farm grassland or into the sewer system). This together with the reported use of community and well water (especially for cleaning the empty poultry houses and equipment) may constitute a risk. Neighbourhood contamination risks may also arise from the practices of the poultry-related businesses. For example, we found

that, at manure companies, manure was dried naturally and stayed unprocessed on the premises for up to four weeks. Furthermore, the waste management of some businesses also contributes to neighbourhood risks. Transmission risks may also arise from transport of materials and products to and from the poultry-related businesses in the farm's neighbourhood. For example, the slaughterhouses that took waste to a rendering plant up to 80 km away and picked birds from as far as 300 km. Neighbourhood-related risks may also be facilitated by the reported pet access to poultry houses and storage rooms on some farms.

On the qualitative risk assessment

In Table 4 we present the outcomes of applying the ranking scheme (Table 1) to the different contact types identified in broiler and layer farms. We found that the contact types deemed most risky comprise the thinning and restocking contacts under category I, almost all human contacts under category II, and the proximity to other poultry farms under category V. Thinning and restocking contacts ranked highly for two reasons namely, their proximity to the birds being close and their frequencies being quite high. On the other hand, the high rank for the human contacts in category II was largely a consequence of their enormous frequency. Generally, category III and IV contacts, due to their combination of category and frequency, are hypothesized to pose a relatively medium overall risk with no clear difference in exposure-risk between layer and broiler farms.

Table 4. Proposed exposure-risk classification for the different contact types for broiler and layer farms based on contact frequency, biosecurity practices and risk category

Risk category	*Contact type*	*Broiler: average number of contacts per year*	*Layer: average number of contacts per year*	*Proposed overall exposure-risk classification based on contact category and frequency: broiler (layers)*
Category I: movement of poultry between farms	Restocking[a]	8	0.61	High (High)[a]
	Thinning	4.8	0	High (Negligible)
Category II: contacts accessing poultry houses	Veterinarian	24.1	1.2	High (High)
	Feed mill technician	24.1	7.8	High (High)
	Hatchery/breeder company technician	2.9	4.1	High (High)
	Repair technician	9.6	1	High (High)
	Inspectors	1.9	1	High (High)
	Vaccination crews[a]	0	0.1	Not applicable (High)[a]
	Catchers[a]	7.7	0.7	High (High)
Category III: contacts accessing	Veterinarian	27	1.3	Medium (Medium)
	Feed mill technician	27.0	8.9	Medium (Medium)

storage rooms	Hatchery/breeder company technician	7.7	4.2	Medium (Medium)
	Repair technician	11.6	1	High (Medium)
Category IV: premises-only contacts	Feed delivery	108.9	64.1	Medium (Medium)
	Fuel delivery	3.2	1	Medium (Medium)
	Bedding supply	4.4	1.7	Medium (Medium)
	Farmer meeting	33.7	0.9	Medium (Low)
	Presence of other animals[b]	6 farms[b]	10 farms[b]	Medium (Medium)
Category V: neighbourhood risks	Proximity to poultry farms[c]	20[c]	37[c]	High (High)
	Proximity to poultry-related businesses[c]	1[c]	2[c]	Medium (Medium)

[a] the risk was adjusted to also cater for the number of people involved during the process, for example, a broiler farm is restocked by only the truck driver and farmer whereas on layer farms, up to 25 people are involved in catching the pullets and delivering them.
[b] Number of contacts per year estimated, the risk is derived based on the number of farms reporting the contact.
[c] Number of contacts per year estimated, the risk is derived based on the number of farms or poultry-related businesses in the 5x5 km square neighbourhood.

Discussion

We conducted an in-depth interview study on the day-to-day activities in the Dutch poultry industry with the aim of identifying all exposure-risks resulting from between-farm contacts (whether proximity related or due to visitors) that are of potentially relevant to AI virus transmission in the industry. Although some detailed results may be specific to the Dutch situation, we expect that many of our findings may be applicable to other countries with a similarly structured poultry industry. On the one hand, the interviewees were selected across all different poultry husbandry types as well as poultry related businesses, in order to collect responses about the full range of day-to-day activities throughout the Dutch poultry industry. On the other hand, the practices identified on the basis of a set of sixty interviewed enterprises might obviously still not be exhaustive.

A similar approach (of interviewing farmers about their farm contacts and the accompanying biosecurity) has been adopted in other recent studies. Examples include Dent et al. [106], Fiebig et al. [107], Vieira et al. [108], Dorea et al. [109], Leibler et al.[110], Burns et al. [111] and van Steenwinkel et al. [112] among others. Our

approach of conducting questionnaire-guided personal interviews had the advantages of obtaining a 100% response rate as well as providing the opportunity for a dialogue between the interviewer and the respondent through which additional information was obtained.

Perhaps surprisingly, the farmers perceived the risk of AI virus introduction by the wild birds accessing water bodies in farm neighbourhoods as being low. Furthermore, they (farmers) had divergent visitor-risk opinions for all visitors (Figure 1). In particular, the result that many farmers attach a low risk to the veterinarians may reflect a relationship of trust. In line with this, most farmers do not force veterinarians to comply with biosecurity protocols (Table 3). One observation relating to the possible actual risk posed by veterinarians is that they were found to make more frequent visits that involved accessing poultry houses and storage rooms than, for example, the catchers (Table 4). A high discrepancy between the reported and GIS extracted numbers of poultry was also found. This information is vital since the attitudes and knowledge expressed may influence the pattern of adherence to biosecurity protocols on the farm.

We identified and categorized several human and non-human between-farm contacts that can promote AI spread. Given that biosecurity is among the main preventive measures against farm contamination through these contacts, it is striking that our findings reveal inconsistency in adhering to biosecurity protocols even against the already 'known' risks (Table 3). The identified obstacles to proper biosecurity practices include absence of facilities, the non-exhaustive protocols and non-adherence or inconsistent application. On adherence, a similar inconsistency was found in other recent studies on biosecurity implementation, for example Racicot et al. [113] and Burns et al. [111] .

The different transmission pathways we hypothesized are deemed relevant for various reasons. Category I pathways (movement of poultry between farms) are important due to the possibility of introducing infected birds on the receiving farms. The infection in transported birds (day-old chicks, pullets or older birds) may go unnoticed for less virulent HPAI strains- for example the H7N7 A/Chicken/Netherlands/2003 [92] that is less virulent compared to, for example, the H5N1 A/Chicken/Legok/2003 [114] - or in case of an LPAI strain. In pullets, LPAI infections can be present without any clinical signs and virus transfer to another farm or geographical area is possible during that time. We note that, although vertical transmission has been reported for turkeys [115], the risk that infected hatching eggs may produce infected day old chicks is considered low because the infected embryo is not likely to survive the incubation process.

The other categories are deemed risky due to the possible direct introduction of infectious material into the poultry house for category II, into the storage rooms for category III and onto the premises for category IV related contacts. The category III related contacts (both human and fomite) may contaminate farm inputs (e.g., feed) if they accessed contaminated material prior to the visit whereas the category IV related contacts may require secondary mechanisms to aid the transfer of infectious material from the compound into the poultry houses. Pets, humans and equipment may provide this link.

The frequency of all the contacts that constitute the category II and III pathways reported is enhanced by the reported multi-site ownership which often comes with increased sharing of labour and equipment as well as the reported exchanges of personnel between farms through visits by the farmers themselves, their family members and the hired personnel. We also note that the reported practice (on some

farms) of not having biosecurity protocols for the delivery-truck drivers and only rely on the fact that they always remain inside their trucks may expose these farms to contamination on (emergency) occasions that may require the driver to get out of the truck while on the premises.

The presence of other animal species (both commercial and pets) might also be relevant to AI introduction. This is because of its associated activities such as the increased number of farm visits through for example, feed delivery and on-site veterinary care. Other than that, there is also the possibility of these animals acting as vectors by transporting contaminated material between locations as well as the possibility of cross-species transmission. AI viruses are known to affect hobby birds and other animal species such as pigs, horses and cats [116-118] and are often less virulent in other species of birds for example Pekin ducks [119]. If infected, some of these species (e.g., pets) have a chance of directly infecting poultry or contaminate feed since they were reported to access poultry houses and storage rooms on some farms.

The several identified neighbourhood-transport related risks may be additionally relevant when long distances are covered thereby extending the geographical range of neighbourhood contamination risks. Examples of long distance transports reported include transports to slaughterhouses and rendering plant. We note that such distances are to be expected for the transports to the rendering plant as there is only one rendering company in the Netherlands that has only two destinations for carcasses.

The risk posed by neighbourhood-related contacts may be controlled by, for example, reducing scavengers through covering the manure storages that were reportedly left open on some of the interviewed farms and/or ensuring that manure does not stay long on the premises as well as ensuring that dead birds are disposed of safely. In addition, since dust acts as a vector on which the infectious material colloids for dispersal, airborne contamination risks could be reduced through installation of dust extraction systems like air scrubbers. Such systems would help in reducing contaminated dust emissions from poultry houses. Moreover, sprinkling oil in the poultry house may reduce the amount of dust emitted, although this method is not very user friendly. Additionally, measures to reduce the dispersal range of the emitted dust such as lowering the vent height as well as measures that reduce the risk of a farm letting in contaminated dust may lower the risks.

In the qualitative risk assessment, we focused on pathways identified in broiler and layer farms. Since the other husbandry types had almost similar contact frequencies, the risk ranking found (for layers and broilers) might not differ significantly in the other types. Layer and broiler husbandry types are the dominant poultry husbandry types in the Netherlands and consequently were the most represented in the interviews. Due to data limitations, we cannot draw firm quantitative conclusions about the actual risks; the rankings made in this study have an indicative, qualitative character. We used the five categories of pathways as a basis for this assessment. Note that, whereas the categorization helps to distinguish different levels of risk that a single contact may pose, the overall risk for a given contact type is a combination of the per-contact risk and the contact frequency. Thinning and restocking both pose a high risk although, unlike thinning, restocking on a broiler farm is done by a small team (mostly the truck driver and the farmer), requires less time and equipment and does not involve intensive handling of animals.

Even though our results reveal that most exposure-risks are almost similar for broiler and layer farms, during epidemics, for example the 2003 H7N7 HPAI epidemic

in the Netherlands [120] and the 2005 H5N2 LPAI in Japan [39], layer farms were more likely to be affected. For the Dutch epidemic, the difference in risk may be explained by the relatively higher density of layer farms compared to broiler farms in the affected regions. We also emphasize that some of the rankings may be altered during epidemics due to movement restrictions.

Since poultry movement between farms poses the highest risk, we sought information about other relevant known contacts to supplement the reported ones. We found that, other than the reported movements during thinning and restocking, poultry movements also occur a few days after restocking when replacing the dead (early mortality) or the small-size birds. They also occur when farms are getting rid of the spent or old hens and/or when there are not enough pullets on the farm. Some farmers also buy spent hens from traders on rare occasions to increase their flock size.

All in all, our in-depth interviews facilitated the identification of several hitherto under- appreciated avenues for AI virus transmission between farms that need to be considered when designing or implementing prevention strategies. The results of this study provide clues on the possible mechanisms of virus transmission relating to the various farm activities and neighbourhood characteristics. The additional mechanisms hypothesized here can be put into consideration when updating the manuals that guide contact tracing during future epidemics.

We have found that there is currently widespread non-adherence to existing biosecurity protocols. Our qualitative risk assessment results should help to prioritize improvements in biosecurity. Our results, including those on farmer opinions, are also relevant for the communication with farmers and poultry-related businesses about practices and risks. We recommend that authorities and sector organizations review the biosecurity protocols and develop (intensified) communication strategies to encourage adherence to these. On-farm facilities designed to help everyday adherence, such as walkways, barriers/fences and warning signs, are currently often missing. Finally, the frequency of risky visitor types should be reduced where possible.

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Chapter 5

Small distances can keep bacteria at bay for days

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In preparation

Abstract: Transmission of pathogens between spatially separated hosts, i.e., indirect transmission, is a commonly encountered problem when fighting epidemics of infectious diseases. In order to improve control strategies against indirect transmission, a better understanding is needed of its underlying routes, which most often remain untraced. We used a novel design to study indirect transmission experimentally and developed a diffusion model to describe the indirect transmission observed. We show that the interplay of diffusion of infectious material and its decay in the environment can explain the large differences in indirect transmission patterns between two different zoonotic bacteria. An immediate consequence is the apparent need to include a distance-dependent delay in transmission; this delay can be predicted by diffusion models. Indeed, a delayed transmission component exists also for the spread of Vancomycin-Resistant Enterococcus in an intensive care unit, and this component disappeared when the environment was thoroughly cleaned. Furthermore, the model allows analysis of the impact of specific bio-security measures against untraced indirect transmission by considering their effects on the diffusion coefficient and the pathogen decay rate.

Indirect transmission, i.e. transmission without direct contact between hosts, is an important mechanism of disease spread in epidemics as has been demonstrated in plants(e.g. [121-123]), in livestock (e.g. [10,42,124-126]) and in humans (e.g. [127-129]). Indirect transmission is very important because control measures can prevent direct contact but it is unclear how indirect contacts can best be avoided. Thus, for example transmission in health care facilities occurs frequently by indirect transmission, in spite of the hygiene measures that are taken.

Better understanding of the mechanisms that underlie indirect transmission is needed to improve effectiveness of bio-security methods to control disease spread. Here we obtain mechanistic insight by studying indirect transmission in controlled experiments and by using mathematical modelling to understand the experimental observations. In our experiments, we concurrently inoculate groups of broilers with two different pathogens and study the indirect transmission of these pathogens to spatially separated susceptible recipients. The two pathogens used have very different decay rates in the environment. The rationale of this approach is that through investigating the influence of the decay rate on indirect transmission, we may improve our understanding of how pathogen-containing particles travel through the environment from sender to receiver. The experimental setup consisted of, in each replicate, inoculated infectious broilers in a centre cage surrounded by ten recipient broilers placed individually in cages at a distance of approximately 75 cm both from the central cage and from each other (Figure S1). All broilers in the centre cage were inoculated with either *Campylobacter jejuni* (*C. jejuni*) or both *C. jejuni* and *Escherichia coli (E. coli)*. The occurrence of indirect transmission events was monitored by a daily collection of cloaca swab samples from all recipient broilers. The experiment ended 35 days post inoculation (p.i.) (see Supporting Online Material (SOM) for full description of experiment [130]). In mathematical models, direct pathogen transmission is usually assumed to occur instantaneously when susceptible and infectious individuals are at the same location at the same time [131-133]. Modelling indirect transmission necessitates inclusion of the transport of infectious material in the environment between hosts, thereby allowing for time delays between pathogen shedding by an infectious

host and subsequent exposure of a recipient host [134,135]. In order to quantify the indirect infection pressure experienced by a susceptible recipient at a specific location at a specific time, the full history of how many infectious individuals were present at particular locations up until the time of interest needs to be taken into account. Here we developed a model in which the transport process was assumed to be diffusion of particles, i.e., infectious material was assumed to move with small random steps [136,137]. One appealing consequence of this simplification is that we do not have to parameterize unobserved individual displacements of infectious material through the environment. Instead, we fit a single parameter (the diffusion coefficient) to the observed pattern, averaging over all transport routes.

Cages with infectious broilers are modelled as an area source of pathogen-containing particles from which diffusion at rate D to the recipient cages occurs. For an area source emitting with strength Q_0 during a time interval $[0, \tau]$, the concentration of viable infectious material at a given location (x,y) at time t is obtained by integrating the point-source solution of the diffusion equation over both space and time taking into account the decay rate (α):

$$S_{\text{cont}}(x,y,t) = \int_0^{\tau}\int_{y_1}^{y_2}\int_{x_1}^{x_2} \frac{Q_0}{4\pi D(t-t')} \exp\left[-\alpha(t-t') - \frac{(x-x')^2 + (y-y')^2}{4D(t-t')}\right] dx'dy'dt'$$

The force of infection (FOI) experienced by a recipient animal is assumed to be proportional to the average concentration across its cage floor area. However, this is true for as long as the concentration is (much) smaller than an "exposure capacity" K [138]. For larger concentrations, the FOI is assumed to be bounded by a maximum equal to βK which is determined, for instance, by limitations in access to and/or uptake of infectious material by recipient animals. This formulation ensures that, even in the limit of negligible pathogen decay, the infection rate will remain finite as required biologically. See SOM [130] for the resulting equation.

The model parameters and their dimensions are listed in Table 1. The parameters that need to be estimated from experimental observations are the diffusion coefficient D, the transmission parameter β_{campy} for *C. jejuni*, β_{coli} for *E. coli*, the exposure capacity K and the decay rates of the pathogens α_{campy} & α_{coli}. The two decay rates are estimated in separate survival experiments (see SOM for full description of experiments[130]), carried out under the same conditions as the transmission experiments. Estimated decay rates were 2.25 day^{-1} for *C. jejuni* and we used zero for *E.coli*, as we observed 100% survival during 100 days. The remaining parameters were estimated using a maximum likelihood estimation approach (see SOM [130]) for the derivation of the likelihood equation).

In the transmission experiments, acquisition of pathogens was detected in 24% of recipients for *C. jejuni* and in 100% of recipients for *E. coli*. The observations are summarized in Figure 1.A key observation was the difference in timing of the first transmission event for the two pathogens (see Figure 1). For E. coli, there is a delay of 4 days and 5 days p.i. to the first transmission event for groups with 5 and 20 inoculated animals, respectively. For C. jejuni first transmission events occurred at day

12 p.i. for the groups with 20 and at 23 days p.i. for the groups with 5 inoculated animals.

In Figure 1 is also shown that our diffusion model for indirect transmission (solid lines without symbols) can explain the difference in the onset of transmission of the two pathogens. The corresponding estimates of the parameters are listed in Table 2. We note that the transport of both C. jejuni and E. coli through the environment is assumed to be governed by one and the same diffusion coefficient. This is motivated by the fact that the broilers inoculated with both C. jejuni and E. coli concurrently excreted both bacteria in the faeces, thus, both pathogens are most probably transported together).

As the two bacteria are excreted in similar amounts, our model fit explains the difference in timing of first infection events in terms of the difference in pathogen decay during transit from sender to recipient. This difference between *C. jejuni* and *E. coli*, in the predicted delay until the amount of infectious material available to recipient animals becomes sufficient to cause infection, is further illustrated by Figure S2. For any given time, the force of infection is higher for the groups with 20 I-animals compared to 5 I-animals but the difference in delays is maintained.

The difference between *C. jejuni* and *E. coli* in terms of amount of infectious material predicted to reach the recipient animals is caused by differences in their decay rates. *E.coli* survives throughout the experimental period while *C. jejuni* only survives for on average 0.44 days. As a result the accumulation of pathogens in the environment at a given location is much slower for *C. jejuni* compared to *E. coli*. The time needed to reach a location and the decay occurring during that time determine the saturation level at that location if the emission of pathogens is continuous at constant rate. Saturation levels reached in our experiments are thus predicted to be lower for *C. jejuni* compared to *E. coli*. Furthermore, the model predicts that there is a limit to the distance that pathogens can reach in substantial amounts. Formulated more mathematically, for every pathogen quantity level there is a maximum distance at which that quantity level can be reached (Figure S3). This distance limit is determined by the decay rate and the diffusion parameter *D,* which depends on the mode of pathogen excretion and manner of transportation. Since the different microorganisms are likely to be transported in the same way, *D* can be estimated independent of the pathogen type.

The combination of animal experiments and modelling carried out here provides new insights in the mechanisms underlying disease transmission as well as new possibilities to quantify effectiveness of infection control measures. The model developed leads to a parameter likelihood that combines the distances travelled and time elapsed since emission. In particular, the diffusion coefficient D describes how fast the disease spreads and the distance it can cover depending on its transport medium (excreta) and external environmental factors (wind, humans, animals and machines). Since the diffusion coefficient D can be modified by external factors, it is a promising candidate for assessing the role of bio-security measures in limiting disease spread. For instance, experiments with safe model micro-organisms (e.g. live vaccines) could be performed to compare estimated values of *D* with and without interventions. Furthermore, the

model predicts that infections with microorganisms with low decay rates can occur at distant locations (long) after the source of infectious material has been removed. This would have important consequences in hospital Intensive Care Units (ICU) where this would imply that removing (or quarantining) a patient colonized with a certain pathogen might not prevent subsequent transmission if that pathogen survives in the environment. This prediction was investigated using data from a study of Vancomycin-Resistant Enterococcus (VRE) in an ICU [139]. In the original study intensified environmental cleaning was associated with reduced acquisition of VRE. Given that newly admitted patients enter the ICU in a clean (and sterilised) bed, we assume that the surfaces immediately surrounding such a patient initially are not contaminated with VRE. Without sufficient cleaning, the further inanimate environment of a patient may still be contaminated with VRE from patients previously occupying the unit. As VRE has comparable survival times [140-143] as *E. coli* and as the relevant distances are comparable to those in our animal experiment, our model predicts a delay in indirect transmission of approximately 4 days. During intensified cleaning the contamination level of the environment would be reduced whenever cleaning removes VRE from surfaces more rapidly than contamination occurs through diffusion. In those situations we expect indirect transmission to be absent. In a new analysis of the ICU data of [139] we found a delayed transmission component in the acquisition of VRE, with a delay of 4 days in periods without intensified cleaning (Fisher's Exact test, $p=0.035$), in agreement with our model prediction (see Supporting Online Material for details of the study and analysis [130]). This delayed transmission component is not observed during intensified cleaning, indicating that it is most probably due to surface-contamination near the patient (Figure S4). Figure 2 shows the cumulative relative number of infected patients per day after admission to the ICU for the undelayed and delayed component. A delay of 4 days implies that – in this ICU – regular cleaning of the environment (at least once a week or more) is enough to counteract diffusive delayed transmission of VRE. This emphasizes the importance of evacuation, cleaning and disinfection measures that are often taken to avoid such transmission. Our diffusion model provides a means to understand and quantify the expected transmission risks and the impact of control measures.

As noted above, indirect transmission is often caused by multiple and difficult to quantify mechanisms. Our results demonstrate that two-dimensional diffusion modelling is a promising approach to describe indirect transmission in a parsimonious manner; with few parameters that can be feasibly estimated. The approach was successful in explaining key features of the indirect transmission of the two bacteria studied here and of the transmission of VRE in an ICU.

Table1. Dimension and description of parameters used in the model.

Parameter	Dimension	Description
S_{cont}	$\#/m^2$	Concentration of pathogen on the time and location of interest
t`	Day	Time of release of the particles
T	Day	Time of interest
(x`, y`)	(m,m)	Location of the source cage
(x,y)	(m,m)	Location of the recipient cage
x_1, x_2, y_1, y_2	M	Coordinates of the source cage
x_a, x_b, y_a, y_b	M	Coordinates of the recipient cage
D	m^2/day	Diffusion coefficient
α	day^{-1}	Decay rate of the pathogen
K	$\#/m^2$	Exposure capacity
β	day^{-1}	Transmission parameter

Table 2. Estimated values and 95 % confidence intervals for the model parameters.

Parameter	Point estimate (95% C.I.)	
	5 I-animals	20 I-animals
D	0.003 (0.002-0.004)	0.0025 (0.002-0.005)
β_{Campy}	0.007 (0.004 - 0.015)	0.015 (0.0053-0.0196)
$\beta_{E.\ coli}$	0.023 (0.0145 - 0.0345)	0.025 (0.016 - 0.037)
K	$1 \cdot 10^{-15}$	$1 \cdot 10^{-15}$

Table 3. Average colonisation rate per period for the baseline situation and the three treatments of the ICU transmission data. A p-value < 0.05 indicates a significant difference between the colonisation rate in period 1 and period 2. p.a. = post admission.

Treatment	Period 1 (day 1-3 p.a.)	Period 2(≥day 4 p.a.)	p-value
Baseline	0.023495	0.050085	0.038
Treatment 1	0.021336	0.003527	0.210
Treatment 2	0.014849	0.014844	0.631
Treatment 3	0.015618	0.010426	0.532

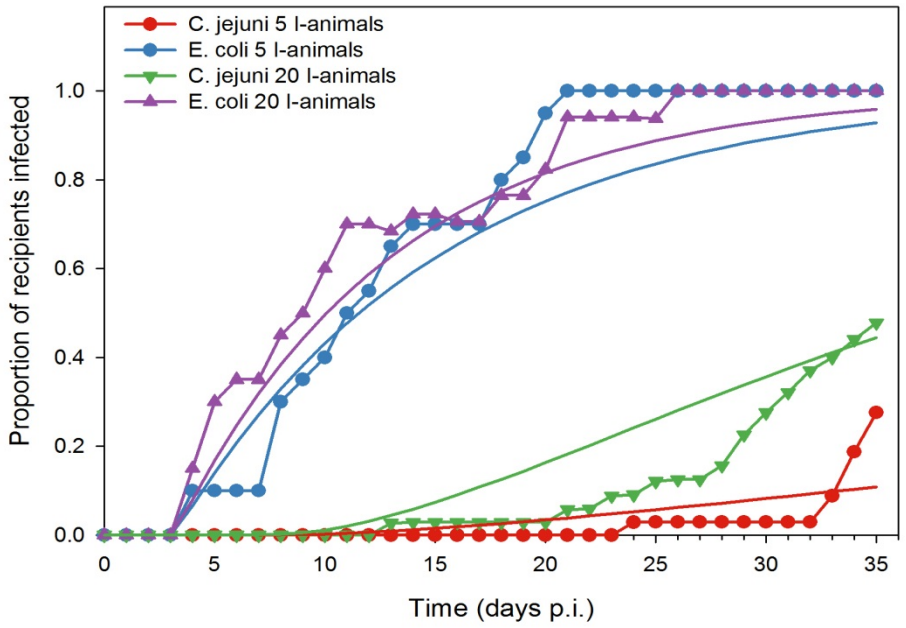


Figure 1. Proportion of recipient animals infected with *C.jejuni* or *E. coli* as function of time since inoculation of the sender animals. In the transmission experiment each experimental room contained 5 or 20 sender animals that were inoculated with either *C. jejuni* or with both *C. jejuni* and *E. coli* and 10 susceptible animals. Curves with circles depict the animals that were infected through indirect transmission with *E. coli*. Curves with triangles depict the animals that were infected through indirect transmission with *C. jejuni.* Solid lines without symbols depict model predictions for that specific treatment. For *C. jejuni* the curves represent the proportion infected of the total number of recipient animals. For *E. coli* the curves represent the proportion infected of those still present on that day, because animals are removed when they are infected with *C. jejuni* (see SOM[130]) p.i. = post inoculation.

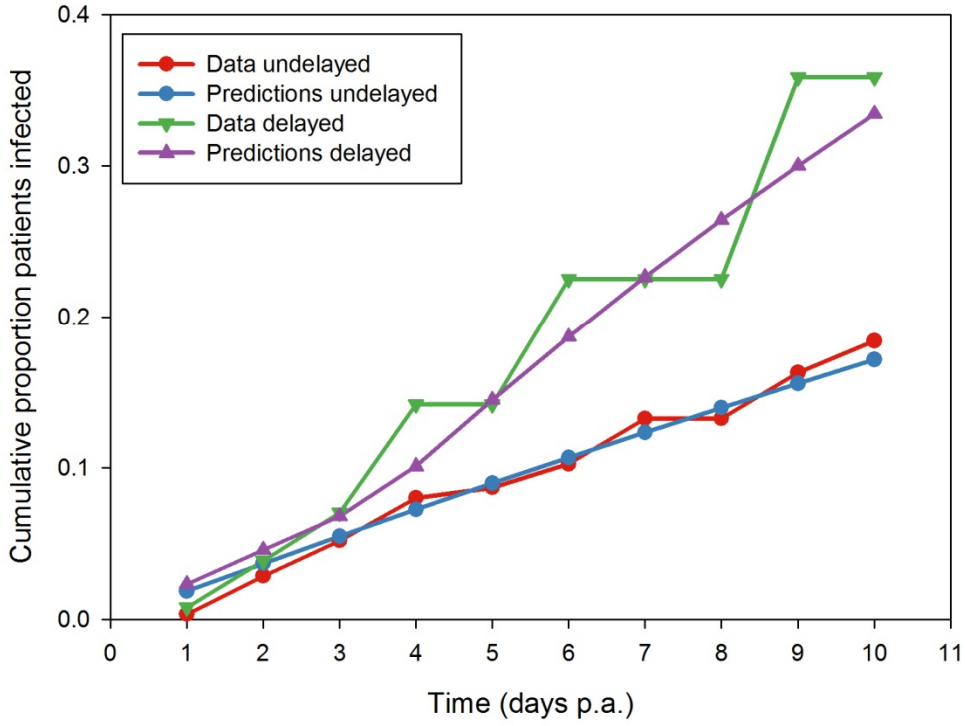


Figure 2. Cumulative proportion of patients infected per day after intensive care admission for the undelayed transmission component and the delayed transmission component. Day 0 is the day of admission.

Supporting Online Material

Transmission experiment

Experimental design

The experiments were carried out on eight groups of broilers. Four groups were inoculated with *C. jejuni* and four groups with both *C. jejuni* and a labelled *E. coli* (see below in the section Inoculation). Two of the four groups inoculated with *C. jejuni* contained five animals and two groups contained twenty animals. The same applied to the four groups inoculated with *C. jejuni* and *E. coli*. See also Table S1 for an inoculation scheme. The inoculated animals were housed together in one cage in the centre of an experimental room (a separate climate controlled room in an experimental facility of the Central Veterinary Institute). Ten susceptible recipient animals were housed individually in cages surrounding this centre cage placed at a distance of 75 cm (see Figure S1).

To track indirect transmission, all source and recipient animals were sampled by means of a cloacae swab (see section on Sampling). These swabs were tested for the presence of *C. jejuni* and *E. coli* (if applicable). Unlike *E. coli* positive animals, if a tested recipient animal was found *C. jejuni* positive, it was considered infected and was immediately removed from the experiment to avoid having to deal with multiple cages contributing to the infection pressure in the analysis. The removed animals were euthanized and cecum was removed for further investigation for the presence of *C. jejuni.*

The experiment ended 35 days post inoculation. All remaining source and recipient animals (that had not been found C. jejuni positive until that moment) were euthanized and cecum was removed and further investigated for the presence of C. jejuni.

Animals and housing

One-day old broilers (type Ross 305) were obtained from a commercial hatchery. At day 7 and day 12 after arrival, cloacal swabs taken from each chick were used to confirm the absence of C. jejuni and nalidixic acid resistant E. coli. From the day of arrival (day 0) until 12 days post-arrival, 180 broiler chicks were housed together in one group. On day 12, the chicks were equally and randomly distributed to eight experimental rooms for the transmission experiment. Four rooms contained five source animals housed together in one centre cage and ten recipient animals individually housed in ten cages surrounding the centre cage as shown in Figure S1. The other four rooms contained twenty source animals housed together in one centre cage and ten recipient animals individually housed in ten cages surrounding the centre cage.

All animals were housed on wood shavings and the drinking water was supplied through a nipple drinking system. In each set-up, the drinking nipples in the cages on the long sides of the area were supplied from one common water container while the centre cage had a separate drinking water supply. This precluded transmission via a shared drinking water system.

Before the start of the experiment, all experimental rooms were cleaned and disinfected with formaldehyde. Subsequently, samples were taken from 12 different areas inside the room to check for the absence of *C. jejuni* and *E. coli*.

Inoculation

For inoculation with *C. jejuni*, the *C. jejuni* strain 356 [144] was used. The strain was freshly cultured in hearth infusion broth (microaerobically, 37°C, overnight) and diluted in buffered peptone water to obtain the intended inoculation dose (± $1*10^6$ Colony Forming Units (CFU)/ml). The precise concentration (CFU/ml) of *C. jejuni* in the administered inoculum was determined by plating on modified cephoperazone charcoal deoxycholate agar (mCCDA) (Oxoid CM 793) with selective supplement (Oxoid CM 155) before and after the inoculation of the animals. Source animals were inoculated 14 days after arrival with 1 ml inoculum.

For inoculation with *E. coli*, a wild-type isolate was used with a point mutation in the gyrA gene, leading to a resistance to nalidixic acid (minimum inhibitory concentration > 64 mg/L). The strain was freshly cultured in normal saline solution (37°C, overnight) and diluted in buffered peptone water to obtain the intended inoculation dose (± $1*10^6$ CFU/ml). The precise concentration (CFU/ml) of *E. coli* in the administered inoculum was determined by plating on MacConkey agar plates with 100ppm naladixic acid before and after the inoculation of the animals. Source animals were inoculated 14 days after arrival with 1 ml inoculum.

Sampling

To track indirect transmission, all animals were tested by means of a cloacae swab. After an inoculated source animal was found positive for *C. jejuni* and *E. coli* on three consecutive days, swabs for those animals were taken weekly instead of daily. For the susceptible recipient animals, swabs were taken once a day throughout the experiment. On days when both inoculated and recipient animals were to be sampled in each group, the recipient animals were sampled first. Swabs were tested within two hours after sampling in the laboratory

Samples were collected using sterile swabs (sterile plain dry swabs, Copan Diagnostics Inc., USA). For *C. jejuni* swabs were directly plated on mCCDA, incubated microaerobically at 41.5°C for 48 hours and examined for the presence of *C. jejuni*. The swab was then placed in Preston enrichment medium (Nutrient Broth no. 2, Oxoid CM0067 with Campylobacter selective supplement (Oxiod SR0204E) and Campylobacter growth supplement (Oxoid SR0232E)) and incubated microaerobically at 41.5°C for 24 hours. After incubation, it was plated on mCCDA and incubated microaerobically at 41.5°C and examined for the presence of *C. jejuni* after 24 and 48 hours. For *E. coli* swabs were directly plated on MacConkey agar, incubated at 37°C for 24 hours and examined for the presence of *E. coli.* The swab was then placed in a normal saline solution and incubated at 37°C for 24 hours. After incubation, it was plated on MacConkey agar plates, which were then incubated again at 37°C and examined for the presence of *E. coli* after 24 and 48 hours.

Hygienic Measures

To prevent animal caretakers from acting as a vector of transmission between stables, strict hygienic measures were used during the entire experiment. Clean overalls were used at every entry into the experimental rooms. A pair of boots was dedicated to each room, cleaned on entering and exiting it by means of wading through a chlorinated bath (Suma Tab D4, JohnsonDiversity).

To prevent direct transport from one bird to the next bird sterile gloves were changed between handling individual animals. Inoculated animals were always sampled last. Note that still the animal caretakers are part of the activities in the stable that can cause the diffusion within the stable.

Survival experiment

Experimental design

A separate survival experiment was carried out with four groups of five broilers each. The broilers were inoculated at age 14 days with C. jejuni and naladixic acid resistant E. coli by gavage. The groups of broilers were placed in cages in which a board was placed as a floor with normal bedding material on top. A group of broilers was put in the cage for either 24 or 72 hours. After this period the broilers were moved into another clean cage with a new board and fresh bedding material. The board floor from the emptied cage was moved from the cage including all bedding material and faeces and taken into an identical experimental room with the same climate conditions as the transmission experiment described above. A wireframe grid with squares of 10 cm x 10 cm was placed over the board. Each day, starting from the day the broilers were removed from the board, a pooled sample of 10 random squares of the grid was taken. This pooled sample was immediately taken to the lab were the number of CFU's of C. jejuni and E. coli in the sample was counted (see section on Sampling for a complete description). In total 22 boards were obtained, 13 boards on which the broilers were placed for 24 hours and 9 boards with broilers placed on for 72 hours. The reasoning for 24 and 72 hours was uncertainty whether a 24 hour period would yield enough faecal material to analyse; after we finished the analysis we found no difference between samples of boards with material from 24 or 72 hour.

Animals and housing

One-day old broilers (type Ross 305) were obtained from a commercial hatchery. At day 7 and day 12 after arrival, cloacal swabs taken from each chick confirmed the absence of C. jejuni and nalidixic acid resistant E. coli. From the day of arrival (day 0) until 12 days post-arrival, 20 broiler chicks were housed together in one group. On day 12, the chicks were equally and randomly distributed into four groups of 5 animals. Each experimental room contained eight cages each measuring 1.5 by 1 meter. On the bottom of the cage, a board was placed with the same dimensions as the cage floor. Wood shavings were put on the boards as bedding material. The drinking water was supplied through a nipple drinking system.

Before the start of the experiment, all experimental rooms were cleaned and disinfected with formaldehyde. Subsequently, samples were taken from 12 different areas inside the room to check for the absence of *C. jejuni* and nalidixic acid resistant *E. coli*.

Inoculation

The inoculation procedure was the same as described for the transmission experiment.

Sampling

For *C. jejuni* for the first seven days each day a sample of the boards was taken by pooling the faeces from ten random 10 cm x 10 cm. squares and one sample from each board on day 14. The faeces inside one square were collected using tweezers to avoid too much bedding material in a sample. The pooled samples were then transported to the laboratory for further handling. In the laboratory, the samples were diluted with 500 ml buffered peptone water and the mixture was homogenized by placing them for 10 seconds in a Stomacher homogenizer (Seward Colworth Stomacher 400®). From the homogenized sample a series dilution was created by diluting 1 ml in 9 ml of normal saline solution for each step. From each dilution, 0.1 ml was plated on a mCCDA plate. The plates were then incubated microaerobically at 41.5°C for 24 hours and examined for the presence of *C. jejuni*. The number of CFU's was counted on the plate that had between 10 and 100 CFU's.

The same procedure was done for nalidixic acid resistant *E. coli,* except that after day 14 every two weeks a sample was taken and each dilution was plated on MacConkey agar with 100 ppm nalidixic acid and incubated at 37°C for 24 hours after which the number of CFU's were counted.

Derivation of the diffusion model

Consider decaying particles diffusing from source of strength U_0 at $x = 0$. The spatial and temporal distribution of the particles is given by Fick's second law. The partial differential equation governing the diffusion process is

$$\frac{\partial u}{\partial t} = D\frac{\partial^2 u}{\partial x^2} - \alpha u,\ t > 0, x \in (0,\infty) \quad (1)$$

where D is the diffusion coefficient (m^2/day), α is the decay rate (day^{-1}), $u(t,x)$ is the concentration at a distance x (m) from the source after time t (day). The initial and boundary conditions are $u(t,0) = U_0$, $\lim_{x\to\infty} u(t,x) = 0$, $u(0,x) = 0$.

Equation (1) solves to

$$u(t,x) = \frac{U_0}{\sqrt{4\pi Dt}}\exp\left[-\alpha t - \frac{x^2}{4Dt}\right] \quad (2)$$

Equation (2) is the solution that describes the diffusive spread along the x-axis i.e., one-dimensional diffusion of a substance from a point source of an amount U_0 released at $x = 0$ at time $t = 0$.

For diffusion on an infinite plane surface i.e., two-dimensional diffusion, the concentration of the diffusing substance at a radial distance r, where in this case $r^2 = x^2 + y^2$, from the source located at the point (0,0), is given by

$$S(t,r) = \frac{U_0}{4\pi Dt}\exp\left[-\alpha t - \frac{r^2}{4Dt}\right]. \quad (3)$$

The solution $S_{\text{cont}}(t,r)$ for a continuous source emitting over a time interval $[0,\tau]$ is obtained by summing up all the contributions of the puffs emitted at the different time points taking into account the length of the diffusion period i.e., for particles emitted at $t' \in [0,\tau]$, the diffusion period is equal to $(t - t')$. The overall concentration at a radial distance r from the source is given by the convolution of the emitted quantity U_0 at time t' and the distribution $S_r(t)$ as $S_{\text{cont}}(t,r) = \int_0^\tau U_0 S_r(t - t')dt'$.

For diffusion over a two-dimensional space from a continuous point source, the distribution of the particles is given by

$$S_{\text{cont}}(t,r) = \int_0^\tau \frac{U_0}{4\pi D(t-t')}\exp\left[-\alpha(t-t') - \frac{r^2}{4D(t-t')}\right]dt'. \quad (4)$$

Replacing the continuous point source with a continuous area source, for example a rectangular cage with as coordinates for the four corners: (x_1, y_1), (x_1, y_2), (x_2, y_1) and (x_2, y_2), the concentration of particles at a given farther away location (x, y) is given by:

$$S_{\text{cont}}(x, y, t) = \int_0^{\tau} \int_{y_1}^{y_2} \int_{x_1}^{x_2} \frac{Q_0}{4\pi D(t-t')} \text{Exp}\left[-\alpha(t-t') - \frac{(x-x')^2+(y-y')^2}{4D(t-t')}\right] dx'dy'dt' \quad (5)$$

where Q_0 is the source strength per unit time per unit area. This approach of extending a point source theory to an area source situation has been described before (for examples, see [137,145,146]). If we have an area recipient for example a rectangular cage with (x_a, y_a), (x_a, y_b), $(x_b, y_a)$and (x_b, y_b)as coordinates of the four corners, we take $\int_{y_a}^{y_b} \int_{x_a}^{x_b} S_{\text{cont}}(x, y, t) dxdy$.

Figure S2 shows a graph of S_{cont} in time, i.e. the amount of viable infectious material per unit area as a function of time for both *C. jejuni* and *E. coli*.

Based on the independent action hypothesis, the force of infection (FOI) experienced by a recipient animal is assumed to be proportional to the average concentration across its cage floor area, which, from equation (5), will tend to infinity for large t. However, even for direct transmission the rate is not infinite [147-149] therefore it is most probably not infinite for indirect transmission. Here we hypothesize that there is a limitation on the concentration to which a recipient animal is exposed. We define that limiting value as the "exposure capacity" K of the animal. It may be governed by, among others, the mechanism of pathogen uptake as well as the accessibility of infectious material in the cage. Consequently, the FOI is taken to be proportional to the average concentration for as long as the concentration is (much) smaller than K but for larger concentrations, it is bounded by K. The mathematical formulation for the FOI with this behaviour is obtained from the logistic growth model theory [138] as

$$\text{FOI} = \beta \int_{y_a}^{y_b} \int_{x_a}^{x_b} S_{cont}(x, y, t) dxdy \,/\, \left(1 + \int_{y_a}^{y_b} \int_{x_a}^{x_b} S_{cont}(x, y, t) dxdy \,/\, K\right).$$

This formulation ensures that, even in the limit of negligible pathogen decay, the infection rate will remain finite as required biologically. These limitations only influence the FOI experienced by a receiving animal; it will not influence the total amount of pathogen that is accumulated at a given location at a given time. The accumulated amount is the quantity which influences the further diffusion in time and space.

We assume that, for any pathogen amount, there is a non-zero probability of infection which increases exponentially fast with increasing pathogen amount. In literature, this is referred to as the dose relationship for a single-hit model [150] or the independent action hypothesis [63].

Parameter estimation

We use the Maximum Likelihood Estimation approach to estimate the diffusion coefficient D and the transmission parameters β from the data obtained in the experiments. Separate likelihood functions for *E. coli* and *C. jejuni* data were constructed because of the difference in experimental procedure i.e., chickens were removed from the experiment upon colonization by *C. jejuni* unlike the *E. coli* colonized ones which were only removed if they also became colonized by *C. jejuni.* The likelihood function for the *C. jejuni* data, L_c is given by

$$L_{\mathrm{c}} = \prod_{i=1}^{S_t}\left(\mathrm{Exp}\left[-\beta_{\mathrm{c}}\sum_{T=0}^{T_{\mathrm{exp}}} S'_{\mathrm{cont}}[T, r_{i0}, \alpha, D, K]\right]\right)\prod_{j=1}^{N_d}\left(\mathrm{Exp}\left[-\beta_{\mathrm{c}}\sum_{T=0}^{\mathrm{dead}_j} S'_{\mathrm{cont}}[T, r_{j0}, \alpha, D, K]\right]\right)$$

$$\prod_{k=1}^{M}\left(\mathrm{Exp}\left[-\beta_{\mathrm{c}}\sum_{T=0}^{t_k-1} S'_{\mathrm{cont}}[T, r_{k0}, \alpha, D, K]\right] \times \left(1 - \mathrm{Exp}\left[-\beta_{\mathrm{c}} S'_{\mathrm{cont}}[t_k, r_{k0}, \alpha, D, K]\right]\right)\right) \quad (6)$$

The likelihood function for the *E. coli* data, $L_{\mathrm{e}}$is given by

$$L_{\mathrm{e}} = \prod_{i=1}^{M}\left(\mathrm{Exp}\left[-\beta_{\mathrm{e}}\left(\sum_{T=0}^{t_i-1} S'_{\mathrm{cont}}[T, r_{i0}, \alpha_e, D, K] + \sum_{j=1}^{i-1}\sum_{T=t_j}^{\mathrm{Min}[t_i-1,\mathrm{culled}_j]} S'_{\mathrm{cont}}[t_i - T, r_{ij}, \alpha_e, D, K]\right)\right] \times \left(1 - \mathrm{Exp}\left[-\beta_{\mathrm{e}}\left(S'_{\mathrm{cont}}[t_i, r_{i0}, \alpha_e, D, K] + \sum_{j=1}^{i-1} S'_{\mathrm{cont}}[t_i - t_j, r_{ij}, \alpha_e, D, K]\right)\right]\right)\right) \quad (7)$$

S_t is the total number of susceptible chickens that escaped from infection throughout the experiment. In the *E. coli* data there are no animals escaping from infection throughout the experiment (S_t=0), which is why the first factor in Eq. (6) has no counterpart in Eq. (7). T_{exp} is the number of days in the experiment. N_{d} is the total number of animals that died due to other causes than removal (during the complete experiment 9 animals died to other causes than removal). dead_j is the day that animal j died due to other causes. M is the total number of transmission events that occurred, t_k is the day that the transmission event occurred. r_{ij} is the distance between the cage of the source chicken(s) j and the receivers cage i, culled_j is the day that chicken j was culled.

The factors in L_{c} (Equation (6)) are described as follows; $\prod_{i=1}^{S_t}\left(\mathrm{Exp}\left[-\beta_{\mathrm{c}}\sum_{T=0}^{T_{\mathrm{exp}}} S'_{\mathrm{cont}}[T, r_{i0}, \alpha, D, K]\right]\right)$ is the probability of escaping infection throughout the experiment for all escapees, $\prod_{j=1}^{N_d}\left(\mathrm{Exp}\left[-\beta_{\mathrm{c}}\sum_{T=0}^{\mathrm{dead}_j} S'_{\mathrm{cont}}[T, r_{j0}, \alpha, D, K]\right]\right)$ is the probability of escaping until the animal died due to other causes, $\prod_{k=1}^{M}\left(\mathrm{Exp}\left[-\beta_{\mathrm{c}}\sum_{T=0}^{t_k-1} S'_{\mathrm{cont}}[T, r_{k0}, \alpha, D, K]\right] \times \left(1 - \mathrm{Exp}\left[-\beta_{\mathrm{c}} S'_{\mathrm{cont}}[t_k, r_{k0}, \alpha, D, K]\right]\right)\right)$ is the probability of getting infected on day t after escaping $t-1$ days. For the factors in L_{e} (Equation (7)), $\mathrm{Exp}\left[-\beta_{\mathrm{e}}\left(\sum_{T=0}^{t_i-1} S'_{\mathrm{cont}}[T, r_{i0}, \alpha_e, D, K] + \sum_{j=1}^{i-1}\sum_{T=t_j}^{\mathrm{Min}[t_i-1,\mathrm{culled}_j]} S'_{\mathrm{cont}}[t_i - T, r_{ij}, \alpha_e, D, K]\right)\right]$ is the probability of escaping infection from the inoculated and the contact-infected animals and $\left(1 - \mathrm{Exp}\left[-\beta_{\mathrm{e}}\left(S'_{\mathrm{cont}}[t_i, r_{i0}, \alpha_e, D, K] + \sum_{j=1}^{i-1} S'_{\mathrm{cont}}[t_i - t_j, r_{ij}, \alpha_e, D, K]\right)\right]\right)$ is the probability of being infected by either the inoculated or contact-infected animals. The estimates for the parameters D, β_{c} and β_{e} are those that maximize the likelihood of observing the data from the experiments given the functions L_{c} and L_{e}. We obtain the

95% confidence intervals for the maximum likelihood estimates D, β_c and β_e using the likelihood ratio test; for each parameter univariate confidence bounds were calculated.

Analysis of the ICU data

The data of Hayden et al. [139], on the spread of Vancomycin-Resistant Enterococci (VRE) in an intensive care unit (ICU) was re-analysed in this study to evaluate if the observed pattern of transmission provides evidence for a delayed/diffusive transmission component. A detailed description of the setup of this study can be found in the original paper.

Briefly, the original study was intended to assess the performance of three different intervention schemes on the spread of VRE. It comprised of four study periods, each with a different (sets of) interventions: a baseline period (Baseline, period 1), a period with intensified environmental cleaning (Treatment 1, period 2), a "washout" period without any specific intervention (Treatment 2, period 3) and, a period with multimodal hand hygiene (Treatment 3, period 4). During the study period, rectal swab samples were taken daily from patients starting on the day of admission throughout the admission period. Cultures for VRE where performed of those swabs.

Improved environmental cleaning (Treatment 1, period 2) involved explaining to housekeepers the importance of environmental cleaning and increased monitoring of housekeeper performance in addition to the actual environmental cleaning. It also involved daily cleaning of ventilator control panels as well as sensitizing nurses and other ICU staff about the problem of VRE and the interventions.

There were a total of 21 ICU beds available for admission of patients throughout the study period. In total, 748 admissions to the ICU were studied and the average duration of stay was not significantly different for the 4 periods.

Using this data, the daily infection rate per person after being admitted to the ICU was calculated, as a function of day-since-admission. Differences between rates of colonisation for two window periods were analysed using a Fisher's Exact-test with the level of significance set at a p-value less than 0.05.

Table S1. Inoculation scheme of the indirect transmission experiment.

Group	Inoculum	Animals inoculated
1	*C. jejuni*	5
2	*C. jejuni & E. coli*	5
3	*C. jejuni*	5
4	*C. jejuni & E. coli*	5
5	*C. jejuni*	20
6	*C. jejuni & E. coli*	20
7	*C. jejuni*	20
8	*C. jejuni & E. coli*	20

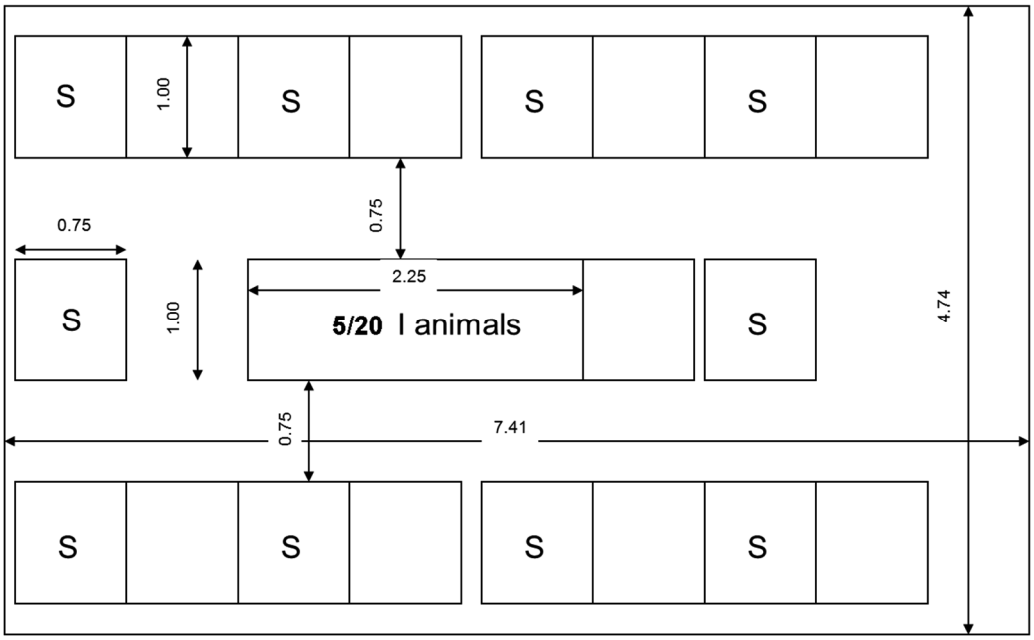


Figure S1. Schematic overview of the housing of the experimental groups of 5 or 20 infectious sender animals and ten susceptible receiver animals. Alongside the arrows distances are given in meters.

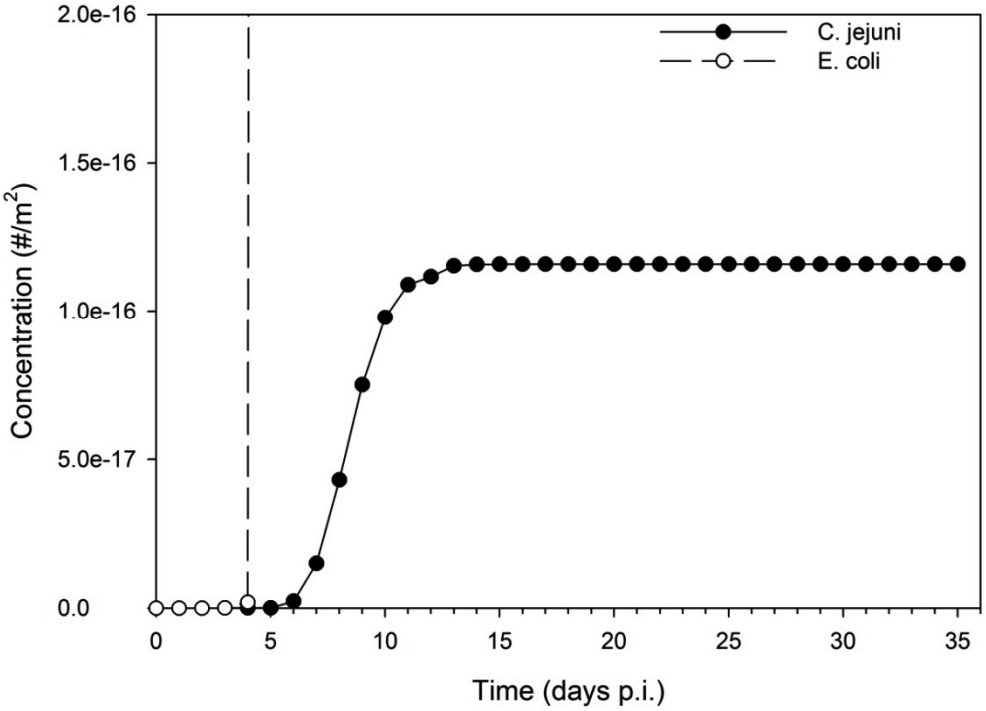


Figure S2. Amount of infectious material per unit area in the recipient cage as a function of time. Note that the curve for *E. coli* quickly rises beyond the scale of this graph because, for E. coli, a decay rate value of 0 was used. Open circles depict the amount of viable *E. coli*. Closed circles depict the amount of viable *C. jejuni*. For the construction of the figure the centre cage was taken as the area source and a cage alongside the centre cage as the recipient source. Parameter values used: D=0.0025 m^2/day, α_{campy}=2.25 day^{-1},$\alpha_{E.coli}$=0 day^{-1}.

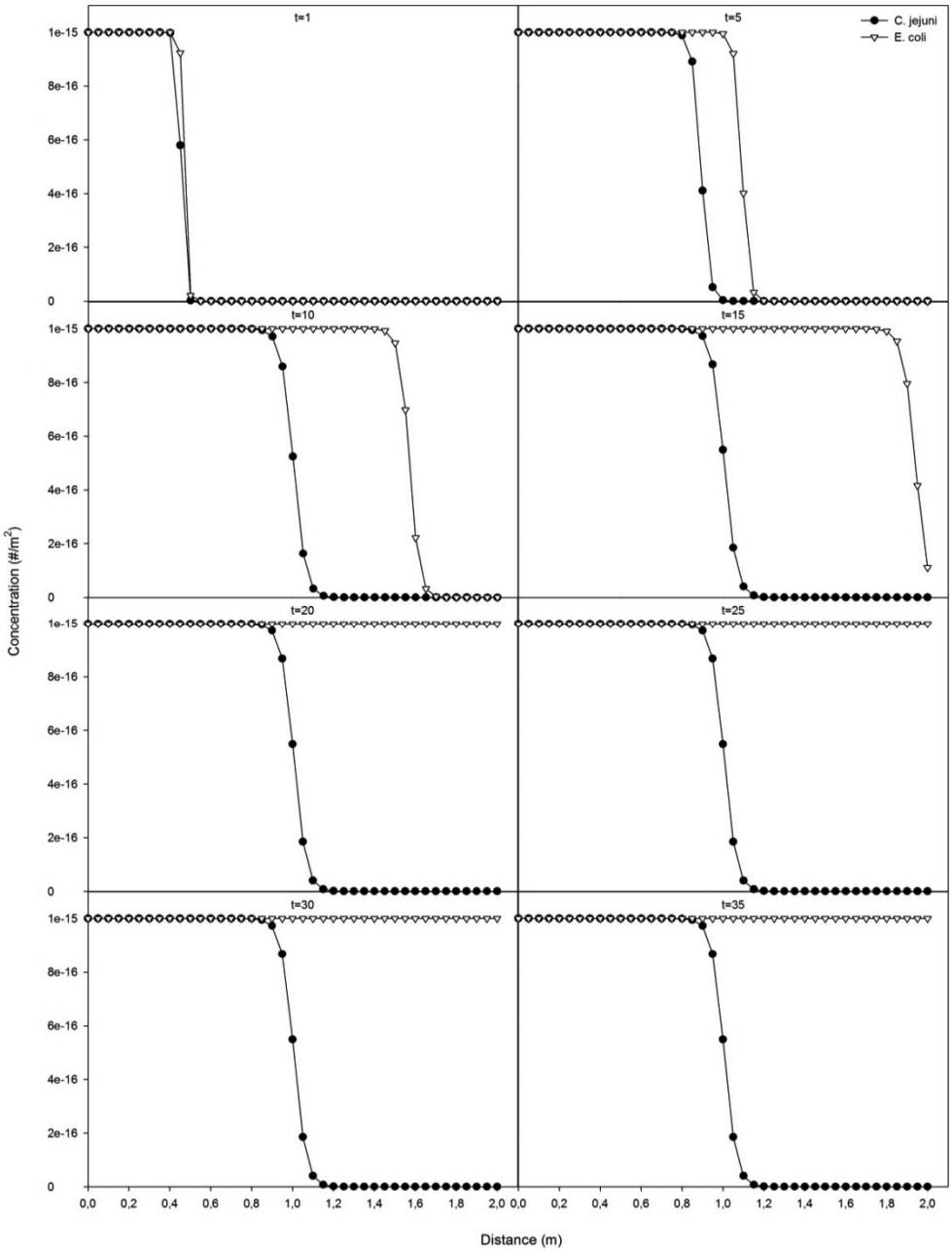


Figure S3. Concentration of viable infectious material as a function of distance from the source. Each panel represents a different time of observation. Parameter values used: D=0.0025 m^2/day, α_{campy}=2.25 day^{-1}, α_{coli}=0 day^{-1},K=1*10^{-15}.

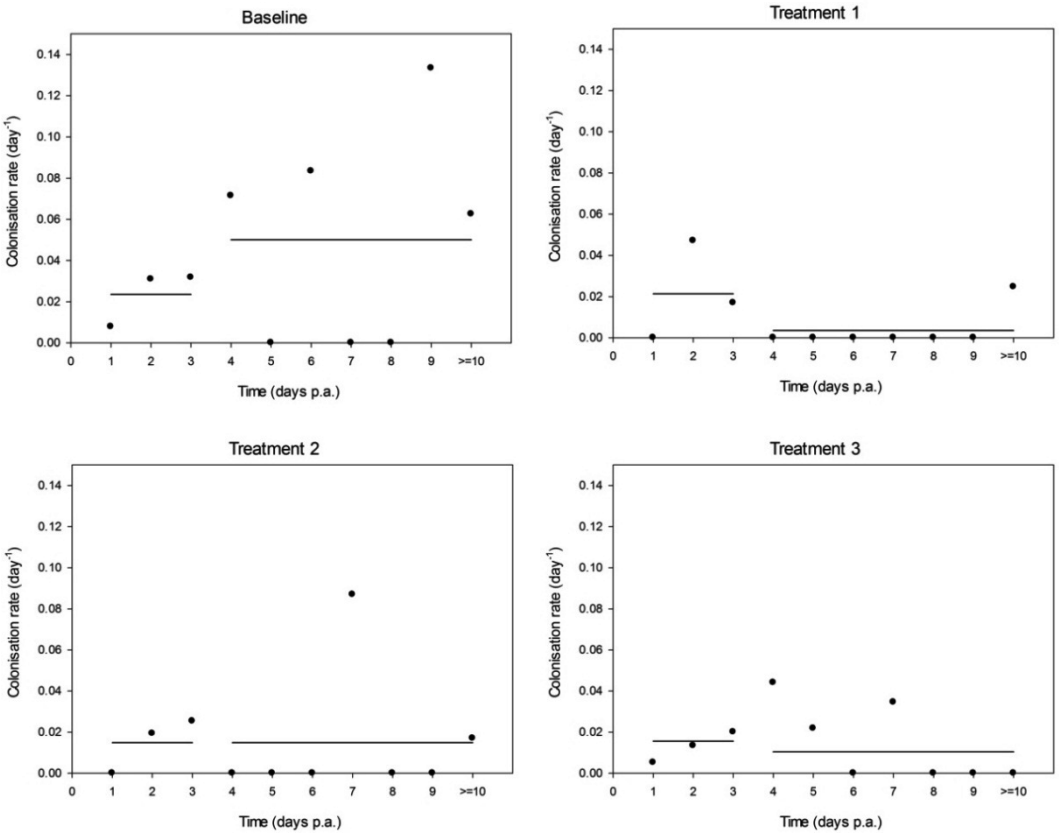


Figure S4. Colonisation rate per day after intensive care admission for the baseline situation and the treatments as defined in Hayden et al [139]. Day 0 is the day of admission. Solid lines indicate average colonisation rate for that period. p.a. = post admission.

Chapter 6

Mechanistic modelling of highly pathogenic avian influenza transmission risk: the role of delayed transmission

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Abstract

In order to improve control strategies for infectious diseases of livestock, one of the epidemic characteristics we need to know is the details on the spatiotemporal mechanisms of spread. From those mechanisms, more accurate information about the distance- and time-dependent transmission risks during epidemics can be generated. Elucidating whether the transmission risk is more dependent on the spatial or the temporal aspects of the epidemic remains a challenge. It requires gaining better knowledge on the transmission process, for example, whether it is direct and instantaneous or indirect and hence delayed. Statistical spatial modelling studies often assume direct (and thus instantaneous) transmission of pathogens between locations and this may lead to an underestimation of the infection hazard at long distances. Here we show that a mechanistic model that assumes diffusion-mediated dispersal of infectious material between locations provides a more accurate approach to quantify the infection hazard. We also demonstrate that incorporating diffusive transport and pathogen decay in modelling disease spread significantly improves the accuracy of the estimates. Based on the Dutch 2003 highly pathogenic avian influenza epidemic data, we parameterised a diffusion-based model using likelihood estimation techniques. The outcome of this model is a transmission kernel that includes indirect and delayed transmission. By comparing it with a kernel that assumes instantaneous infection, we found that assuming diffusion-like transmission improves the model fit and leads to a higher infection hazard at longer distances.

Keywords: diffusion; avian influenza; mathematical modelling; risk maps; transmission kernel

1 Introduction

Livestock disease epidemics caused by Classical Swine Fever virus, Foot and Mouth Disease (FMD) virus and Highly Pathogenic Avian Influenza (HPAI) virus have severe consequences [12,151,152]. These include high mortality and morbidity rates, economic losses accruing from lost stocks and market as well as being a threat to human health for HPAI. To prevent these epidemics, we need effective control strategies. Yet we lack the knowledge about the most important transmission mechanisms such as indirect transmission between farms. In conformity with this, we need better information to be able to calculate more accurately what the impact of control will be by basing on mechanistic model outcomes to extrapolate to new epidemics. Here we seek information for those measures that are based on space: 1) where neighbourhood culling or prophylactic procedures (i.e., interventions intended to prevent spread) have to be applied (for example, the risk maps of Boender et al. [10]) and where not, 2) in what radius around an infected farm vaccination rings can best be located.

We now know general spread patterns such as the distance-dependent probability of farm infection (i.e., transmission kernels) for some epidemics. For example, transmission kernels for the 2001 FMD epidemic in UK [48], the Dutch 2003 H7N7 HPAI epidemic [10] and the Italian 1999 H7N1HPAI epidemic [41] have been estimated. Results from those studies provided the much wanted first step towards understanding the 'neighbourhood' transmission (i.e., termed as such because geographical proximity was found to be a key determinant of transmission risk [47,153]) of the viruses during those epidemics. Their results have since been used to guide

further studies that have deepened our understanding of the mechanisms of spread between locations (for an example on FMD see[154] and for HPAI examples, see [41,106,155,156]). We note however that both studies on HPAI assumed instantaneous infection−only the farms that are infectious on a given day contribute to the infection hazard experienced by the susceptible farms on that day. This assumption can be relaxed by allowing for the possibility of delayed transmission since pathogens may take some time to travel between locations.

In line with the proposed assumption, van Bunnik et al. [157] observed a delayed transmission of bacterial colonisations between spatially separated animals in an experiment. A diffusion-based model incorporating pathogen decay was successful in predicting the observed delay. In a field situation, assuming diffusive transport of infectious material implies that the infection hazard experienced at any given location on a given day is a consequence of all the previous infectious farms up until that day. Since the total infection hazard is conserved whenever different models are fitted to the same data, the only differences will be in the hazard distribution over distance and time.

We introduced a mechanistic description for a multi-stage pathogen dispersal process in the spatiotemporal analysis of part of the Dutch 2003 H7N7 HPAI epidemic data. The hypothesis about a possibility of a multi-stage dispersal process was partly born out of the fact that only 7% of observed transmission during that epidemic was explained by the traced contacts [105] and partly out of the findings of van Bunnik et al.[157]. The proposed model assumes diffusive transport of infectious material−hence implicitly introducing a possible time lag between release of infectious material at the source farm and the occurrence of a secondary infection. By this, we explore the effect of incorporating multi-step pathogen transport and pathogen decay on the distance- and time- dependent transmission pattern during livestock epidemics. Depending on the value of the diffusion constant of the model, delayed transmission becomes an important feature of the proposed model. The approach proposed here provides a way to learn more about the possible mechanisms especially those that may underlie the spread of infectious material between farms.

2 Materials and methods

2.1 Data

We used the 2003 Dutch poultry database to determine the spatial location of all poultry farms in the region studied. For the epidemic-related data, we used part of the data collected during the epidemic which included detailed information about all the culled farms such as culling dates and the farms' ultimate disease status. Each infected and culled farm i is associated with a culling date $t_{i,\text{cull}}$, an infectious date t_i and a location $r_i = (x_i, y_i)$ in Cartesian coordinate system. As in [10], we also assumed the day of infection to be six days before the first day of mortality increase and assumed two days for the latent period i.e., a farm is infectious two days after it is infected. For details on how the data was obtained and the formats in which it was prepared for the analysis, see[10].

2.2 Modelling approach and parameter estimation

We used part of the epidemic and location data of Dutch 2003 H7N7 HPAI epidemic and a maximum likelihood estimation technique to estimate the (generic) transmission rate β and diffusion coefficient D for the Limburg province. We only used part of the

data because of the intensiveness of the computations involved in dealing with the complete dataset. In this analysis, we made a simplifying but necessary assumption that the geographical borders served as epidemic borders implying that the epidemic in Limburg province was independent of the situation in other provinces. This is likely to be a good approximation given the evidence that only between one to three outbreaks in this province were likely caused by transmission from the other affected areas [97,100].

Considering HPAI virus transmission from an infected farm i to a susceptible farm j, the (Euclidean) distance between farm i and farm j is given by $r_{ij} = |r_i - r_j|$. The contacted susceptible farm j may become infected at a later time t_j, or may escape infection throughout the epidemic until day t_{max} or until when it was preventively culled on day $t_{j,\text{cull}}$. Let $K(\tau, r_{ij})$ define the spatiotemporal transmission kernel over one time unit, where τ is the time of interest. The kernel describes the distance- and time-dependent scaling of the probability of 'contact' between farms i and j by any mechanism. We adapt the kernel developed from a 2D-diffusion of infectious material from a point source model [136,145,157,158]. The model is based on diffusion of infectious material that is emitted from a continuous point source, in this case an infectious poultry farm. At time τ, the contribution of a continuously emitting source farm i emitting at times $t' \epsilon [t_i, t_{i,\text{cull}}]$ is given by

$$K(\tau, r_{ij}) = \int_{t_i}^{\min[t_{i,cull}, \tau]} \frac{1}{4\pi D(\tau - t')} \exp\left[-\alpha(\tau - t') - \frac{{r_{ij}}^2}{4D(\tau - t')}\right] dt', \quad (1)$$

where α is the decay rate of the pathogen in the environment per day. Following the approach described in [10,159,160], we define the force of infection on a susceptible farm j at time τ as

$$\lambda_j(\tau) = \sum_{i \neq j} \beta \times K(\tau, r_{ij}) \times I_{ij}(\tau - t_i), \quad (2)$$

where $I(\tau - t_i) = \begin{cases} 1, \text{ if } \tau - t_i \geq 0 \\ 0, \text{ otherwise} \end{cases}$ is an indicator function for the infectivity of farm i.

The probability that farm j is infected at time t_j is obtained from the force of infection as

$$p_j^{\text{infection}}(t_j) = 1 - \exp[-\lambda_j(t_j)], \quad (3)$$

and the probability that it escapes infection up to time $t_j - 1$ is given by

$$p_j^{\text{escape}}(t_j - 1) = \exp\left[-\sum_{s=t_i}^{\min[t_{i,\text{cull}}, t_j - 1]} \lambda_j(s)\right]. \quad (4)$$

The farm escapes infection throughout the epidemic (i.e., until time t_{max}) or until it is culled (i.e., at time $t_{j,\text{cull}}$) with probability

$$p_{m;n}^{\text{escape}}(t_{j,\text{cull}} \text{or } t_{\text{max}}) = \exp\left[-\sum_{s=t_i}^{\min[t_{i,\text{cull}}, t_{j,\text{cull}} - 1 \text{ or } t_{\text{max}} - 1]} \lambda_j(s)\right]. \quad (5)$$

The individual probabilities given above are combined to give a likelihood function L as

$$L = \prod_{m \in \text{escaped_culled}} p_m^{\text{escape}}(t_{m,\text{cull}}) \prod_{n \in \text{escaped_unculled}} p_n^{\text{escape}}(t_{\max}) \prod_{j \in \text{infected}} \left(p_j^{\text{infection}}(t_j) \times p_j^{\text{escape}}(t_j)\right). \quad (6)$$

Equation (6) is then Log-transformed and used to estimate the parameters D and β using the maximum likelihood estimation approach.

2.3 Estimating the dispersal range

From the estimated diffusion coefficient D, we determine the Mean Square Displacement (MSD). This is obtained from the second moment of the diffusion model[161] using

$$\text{MSD} = \int_{-\infty}^{\infty} r^2 \times \frac{1}{4\pi Dt} \text{Exp}\left[-\alpha t - \frac{r^2}{4Dt}\right] dr, \quad (7)$$

where $r^2 = x^2 + y^2$. This simplifies to $4e^{-t\alpha}Dt$ where t is time in days. The square root of the MSD gives the average distance travelled by infectious particles in a given time.

2.4 Model validation, comparison and generation of risk map

We investigate the effect of the differences in assumptions made about the transmission process by comparing the estimated infection hazard from both models and their fit to the epidemic data. Comparing the full spatiotemporal transmission kernels of the previous and current models provides a way to see in detail the differences in how the two models distribute offsprings to parent infections, i.e. "who-infected-who". In addition, one can obtain a less detailed comparison of only the distance dependencies after integrating over time.

The transmission kernel based on the assumption of instantaneous transmission proposed by Boender et al. [10] is of the form

$$K(r) = \frac{h_0}{1 + \left({}^{r}/_{r_0}\right)^{\alpha}}. \quad (8)$$

Both models, i.e. the instantaneous transmission in Equation (8) and the diffusion-based model in Equation (1) are parameterized by fitting each of them to the 2003 H7N7 HPAI epidemic data and poultry farm location data for Limburg province. Other than the realism of its underlying assumption on transmission, the other way of testing the added advantage of using a mechanistic model in improving the predictive power of spatial models can be attained through a comparison of the new model's distance- and time-dependent predictions with those from a previous model that assumes instantaneous transmission.

We note that since both models are parameterized using the same data, they should have similar epidemic characteristics most notably the reproduction number. As described in Boender et al. [10], this reproduction number R_i is given by

$$R_i = 2\pi \int_0^{\infty} \rho_i(r) P(r) r dr \quad (9)$$

and by assuming a uniform farm density ρ, it simplifies to

$$R = 2\pi\rho \int_0^{\infty} P(r) r dr. \quad (10)$$

Upon integrating over time, we compute the distance-dependent contribution to R for both modelling approaches as well as generate risk maps using the threshold phenomenon of the reproduction number R_i for farm i. In generating these maps, each

farm i is categorised into any of the two risk categories namely, high risk if R_i > 1 and low risk if R_i < 1. As was done in Boender et al.[10], $R_i$is estimated by taking into account the actual farm distribution as

$$R_i = E\left[\sum_{j \neq i} P(r_{ij})\right]. \quad (11)$$

The risk maps obtained using the two modelling approaches are compared on the basis of where they predict the most risky areas of the epidemic to be, in relation to the actual/observed epidemic. We also compare the predictions of both kernels based on the individual distance-dependent probability of infection $P(r)$, the predicted number of new cases and the distance-dependent contribution to the reproduction number $rP(r)$.

3 Results

Re-parameterizing Equation (8) based on only the epidemic data from Limburg province yielded the following parameter estimates (point estimate (95% CI): h_0 = 0.0019(0.0009 – 0.0075), r_0 = 4.3(1.0 – 8.0) and α = 2.8(1.49 – 5.31) (compared to h_0 = 0.002(0.0012 – 0.0039), r_0 = 1.9(1.1 – 2.9) and α = 2.1(1.8 – 2.4) based on the full epidemic data [10]). For the diffusion-based model, taking HPAI virus survival of 14 days, i.e. a decay rate $\alpha = \frac{1}{14}$ day^{-1} [99], we estimated the diffusion coefficient D = 22.681 km^{2}day^{-1} and the corresponding β = 0.0526 day^{-1}. The average daily dispersal range was calculated to be 10.173 km day^{-1}.

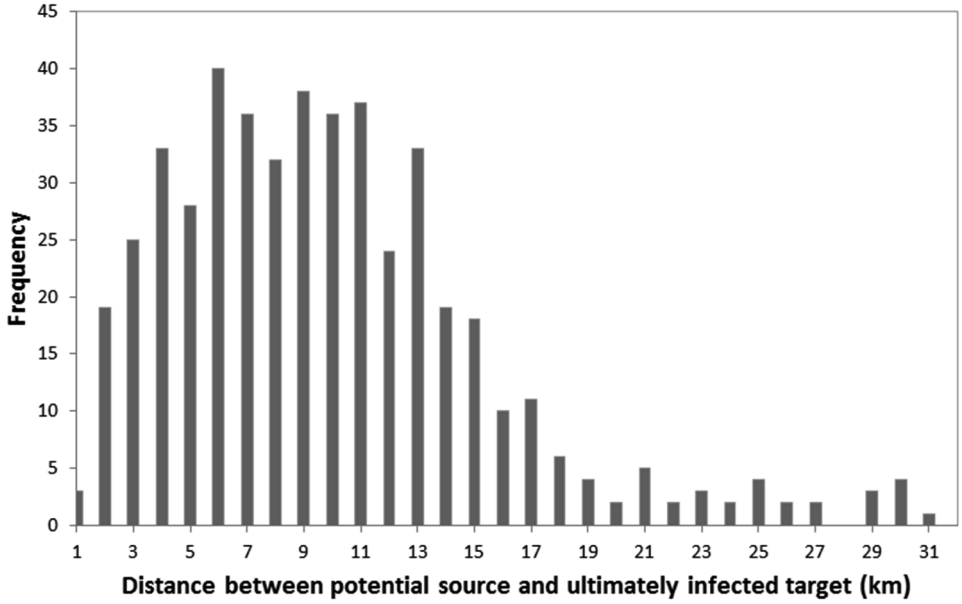


Figure 1. Distance distribution of potential transmission events. It is obtained by extracting distances between all infected farm pairs (A, B) where A was infectious

before B got infected. It gives an approximate scaling of the probability of infection with distance.

Figure 1 presents the distance distribution of potential transmission events and it reflects the distance-dependent distribution of the number of new cases. The farthest distance between any two cases was 31 km while the smallest was 0.5 km. We also observe that more than 75% of potential transmission events occurred beyond 5 km. In Figure 2, we present the estimated distance-dependent probabilities of infection by the two models. We observe that the estimated transmission probabilities from the diffusion-based model are lower than the instantaneous- transmission model at all distance below 7.3 km, the trend is reversed beyond this distance.

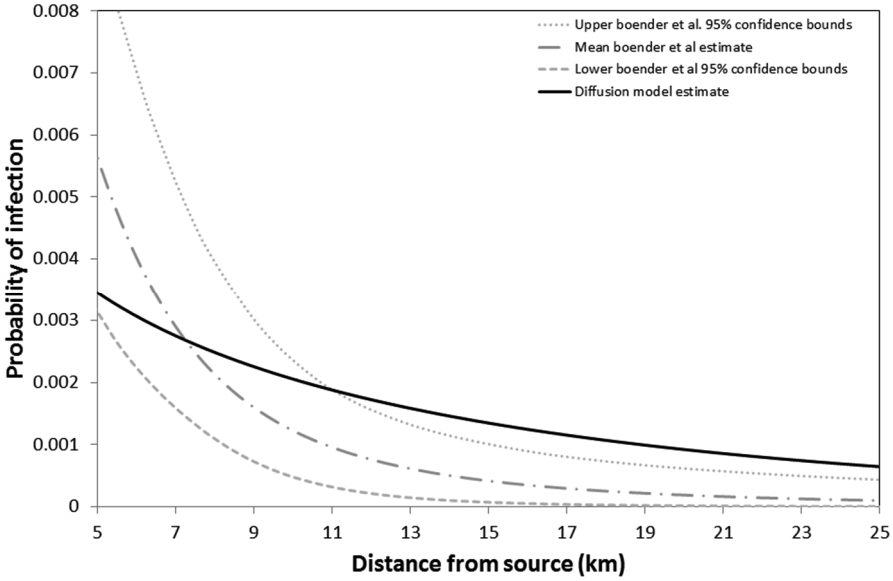


Figure 2. Distance-dependent probability of infection (averaged over all sources) obtained using the diffusive transport model and the instantaneous model (with its 95% confidence bounds). In order to clearly see the pattern at long distances, the x-axis is set to start at 5 km.

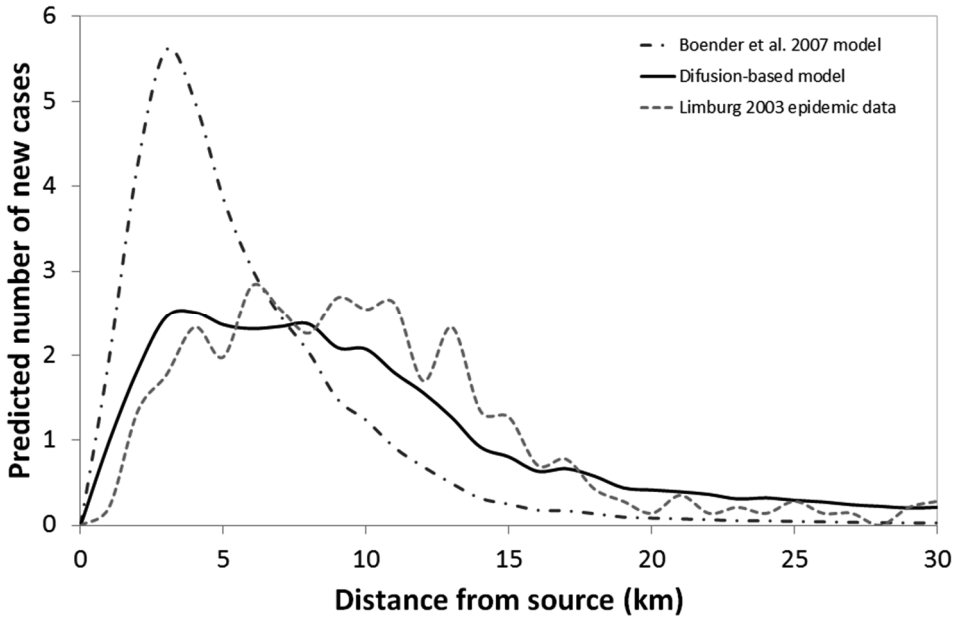


Figure 3. Predicted number of new cases as a function of inter-farm distance. Obtained by multiplying the probability by the total number of farms within a given distance band. The number of new cases observed during the epidemic is obtained by re-scaling the frequency of potential transmission events (Figure 1) to equal the number of cases predicted by each of the two models.

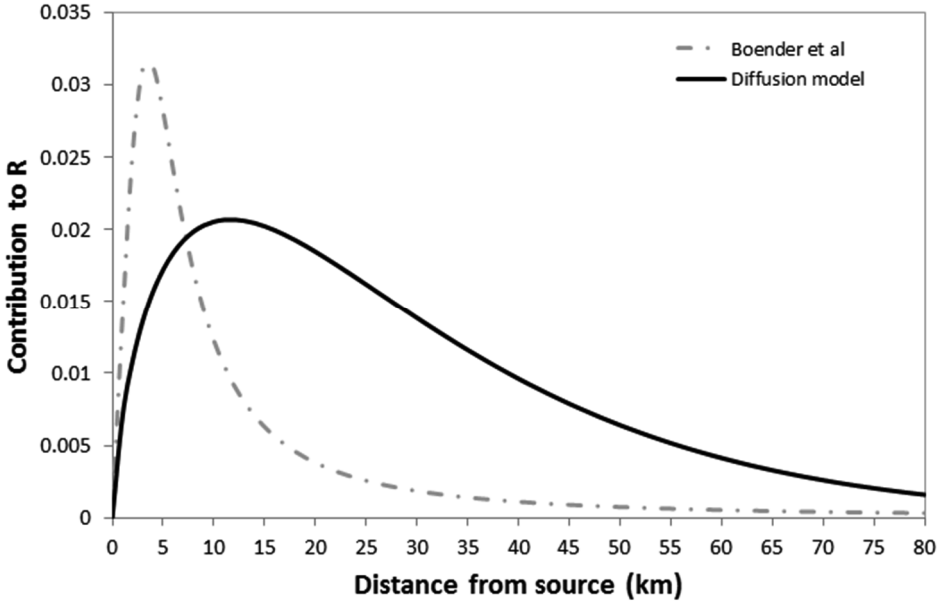


Figure 4. The distance-dependent contribution to the reproduction number. This is obtained by multiplying the probability of infection with distance.

The predicted and observed number of new cases at different distances is presented in Figure 3 and the predicted distance-dependent contribution to the reproduction number R (i.e., by assuming uniform farm density) is presented in Figure 4. In relation to the distance-distribution of estimates, Figures 3 and 4 show a similar trend as that depict in Figure 2; the instantaneous model predicts more new cases (Figure 3) and bigger contribution to R (Figure 4) at short distances and vice-versa at long distances. More importantly, Figure 3 reveals that the distance-distribution of new cases predicted by the diffusion-based mimics the observed pattern during the epidemic.

Figure 5 presents the risk maps for the study area i.e. Limburg province. These maps categorise the province into high and low risk areas of epidemic spread with high risk areas being those in which a substantial number of farms have potential of causing more than one new infection (i.e., R_i > 1) and low risk areas being the vice-versa. A comparison of Figure 5 with Figure 1 of Boender et al. [10] that shows the distribution of infected farms during the epidemic reveals that both modelling approaches are in close agreement with the actual epidemic data in terms of the locations of outbreaks.

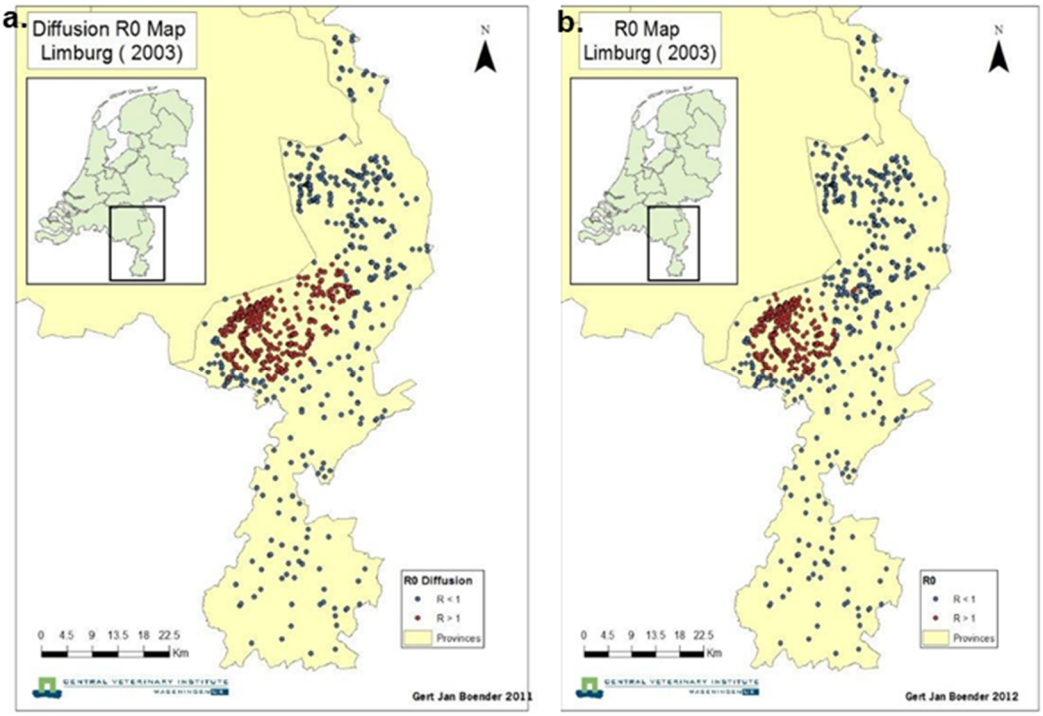


Figure 5. Risk map for HPAI spread during the epidemic. The red dots represent farms that have a potential of infecting more than one susceptible farm (R_i > 1) while the blue dots represent farms which cannot infect more than one susceptible farm (R_i < 1). Panel a is based on the estimation from the diffusion-based model and panel b is based on the Boender et al. (2007) kernel and both kernels predict the same high-risk area.

4 Discussion

It is a common practice to assume instantaneous transmission when studying disease spread between locations. However, we hypothesize that there is likely to be a delay in transmission (thereby non-instantaneous) as a consequence of travel-time requirement for the pathogen. We develop a mechanistic description of a multi-stage pathogen dispersal process between farms as a tool to assess the possible role of delayed transmission in pathogen spread. This concept is relevant when analysing space- and time- related characteristics of an epidemic. We apply spatiotemporal modelling techniques to part of the Dutch 2003 HPAI H7N7 epidemic data and compare the estimated infection hazard from an instantaneous transmission-based model with that of the mechanistic model.

We approximated the dispersal process of infectious material by a step-by-step diffusion between locations. The modelling approach adopted allowed for a parsimonious estimation of a single mechanistic parameter, i.e. the diffusion coefficient as 22.681 $km^2 day^{-1}$. Through the estimation of the average dispersal range (here

estimated from Equation (7) as 10.173 km day^{-1}), the design and implementation of control strategies can be guided. Note that the (generic) transmission rate β (estimated to be 0.0526 day^{-1}) captures quantitative details about the emission, inoculation/exposure route, and dose response processes.

The quantitative distance-dependent patterns obtained reveal striking differences in the performance of the instantaneous transmission and diffusion- based models. Estimates from instantaneous model are consistently lower than those from the diffusion-based model (Figures 2, 3 and 4) at long distances. The risk maps shown in Figure 5 reveal that the diffusive transport based model, as does the instantaneous model, conserves the basic characteristics of the epidemic, yielding some confidence that it could be reliably used to assess the performance of (and hence facilitate the design of new) intervention strategies. For example on how this can be done, see [10].

For purposes of extrapolation of modelling study findings to a field situation, we argue that the best model is the one that is based on correct assumptions and not necessarily the one that fits better to the data. Nonetheless, we found that the predicted number of new cases based on the diffusion-based model has an almost similar pattern as the adjusted frequency of the potential infection events observed (Figure 3). This finding provides evidence to suggest that a diffusion-based model performs better than the original instantaneous transmission model. Therefore, we reliably conclude that instantaneous models tend to underestimate the infection hazard experienced at long distances. This underestimation is a consequence of ignoring the travel-time required for infectious material to disperse between distant units, in this case farms. For short distance transmissions, assuming instantaneous transmission may still give a good approximation because the travel time required for the infectious material may be negligibly small.

We note that analysing the full dataset proved to be prohibitively intensive computationally. For that matter, we performed the analysis using data from Limburg province although the epidemic affected three other provinces namely, Gelderland, noord-Brabant and Utrecht provinces. We acknowledge that a complete analysis would probably give better results and more reliable estimates for the assessment of the performance of intervention strategies.

Nevertheless, through a mechanistic description of the dispersal process, we have been able to generate more insights into possible mechanisms that may underlie the observed between farm spread of HPAI. The additional knowledge gained may help to improve predictive modelling to, for example, assess the impact of control measures on the severity of infectious disease epidemics. With the new approach, we could assess the effect of the 'post-culling' contribution of once infectious (but already culled) farms to the infectious pressure experienced at the susceptible farms throughout the epidemic. More to that, the effect of pathogen decay on the spatial extent of spread may also be assessed directly and the outcomes be used to improve control strategies.

All in all, the proposed diffusive transport model fits better to the epidemic data than the original model that assumed instantaneous transmission. The mechanistic approach of the diffusion-based model gives an insight into the possible mechanisms of pathogen spread. Incorporating delayed transmission redistributes but conserves the total infection hazard over distance. We conclude that delayed transmission is an important phenomenon that needs to be catered for when studying disease transmission between locations/farms. This concept may have a direct influence on how to analyse and interpret epidemic data. For example, it highlights the need to

incorporate an extended infectiousness beyond the culling day of an infectious farm, although the contribution may decrease as a function of time since culling of the source. This extended infectiousness should be considered when designing control strategies against future infectious disease epidemics, especially those involving pathogens that are relatively stable in the environment.

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Chapter 7

General discussion

Introduction

Epidemics involving some World Organisation for Animal Health (OIE) listed diseases such as Highly Pathogen Avian Influenza (HPAI), Classical Swine Fever (CSF) and Foot and Mouth Disease (FMD) continue to cause havoc worldwide[162]. Their routes/mechanisms of between-farm spread are not clearly understood. However, due to the distance-dependence of their transmission risk, they are often referred to as 'neighbourhood' transmission [10,47,153,163]. Some of the currently recommended strategies seem ineffective due to difficulties in implementation (e.g., for quarantine and 'stand still'), labour intensity (e.g., vaccination) and others are deemed unethical (e.g., preventive culling) due to the way the affected animals are treated. Lack of knowledge affects the much needed design of new control strategies as well as the improvement on the existing ones.

The main aim of this thesis was to gain more insight into the mechanisms underlying neighbourhood transmission of livestock diseases during epidemics. To achieve this, mathematical modelling, statistical techniques and field study (or questionnaire survey) and laboratory experiments were used. With the insight gained, new approaches that specifically target the newly hypothesized mechanisms can be developed. The approaches and findings in this thesis can be applied to study the dynamics of livestock diseases in which neighbourhood transmission is known to occur.

Summary of thesis findings

With the help of mechanistic models, the contribution of the windborne route was quantified in form of distance-dependent probability of HPAI transmission between farms by this route. A comparison of the result with the transmission kernel estimated earlier for the same epidemic [10] revealed that close to 24% of all new infections within 25 km could be explained by the windborne route (Chapter 2). In Chapter 3, the contribution of 'active' contacts traced during the epidemic and the (modelled) unknown contacts was quantified by estimating their per-contact probabilities of HPAI virus transmission and the plausible number of new cases they caused during the epidemic and all contacts were found to have made a substantial contribution during the epidemic. Among the traced contacts, egg transports were found to have transmitted the virus for one in every three visits, whereas the crisis organisation contacts were only able to transmit the virus for one in approximately one thousand visits. Around 93 % of the new infections were attributed to the 'unknown' contacts (Chapter 3). The dominancy in contribution of the 'unknown' contacts to the number of new infections highlights the presence of yet-to-be appreciated transmission mechanisms that may underlie these routes. In Chapter 4, the information about the contact patterns and biosecurity protocols obtained from an interview study involving the stakeholders was used to infer about potential transmission pathways. Movement of animals during restocking, thinning and spiking were some of the highest-risk practices on farms and biosecurity breaches were reportedly common (Chapter 4). In pursuit of a more parsimonious approach to understanding indirect transmission of pathogens between locations, a diffusion model that describes the step-by-step dispersal of infectious material was developed (Chapter 5) and applied to the Dutch 2003 epidemic data (Chapter 6). This model approximates the pathogen transportation process by a diffusion process, i.e. a random walk governed mainly by the diffusion

coefficient. This model has the advantage that only one parameter (the diffusion coefficient) needs to be estimated. In addition to that, it was found to fit better to epidemic data than earlier models based on instantaneous transmission. From this modelling approach, new insights such as the possibility of delayed transmission are gained.

Direct or indirect transmission

The spread of pathogens between locations is often categorized into direct and indirect transmission. Direct transmission of livestock diseases entails routes such as transportation of animals between farms. During epidemics however, bans on movement of live animals may reduce the risk of direct transmission of diseases. This implies that contamination of personnel and fomites may remain the known principal way that infection spreads during epidemics. The role of indirect transmission in the spread of livestock diseases [15,42,125,126,134,164] and human diseases [127,165] has been studied although its mechanisms are difficult to control since a good proportion of them may go unnoticed. More efforts are needed to identify and gain more insight into the factors that promote these routes. To this effect, studies need to be conducted as a means to gain more insight in the contribution of environmental transmission to the between-farm spread of pathogens.

The role of the windborne route in avian influenza spread between farms

The windborne route has been implicated in the spread of livestock diseases such as FMD [50-53,166,167], Aujeszky's disease[54] and the role of aerosols in the transmission of other pig diseases has been assessed [168]. In the case of HPAI, mixed opinions about its significance are evident. For example, it was concluded that aerosols and windborne contamination are not important in the spread of infection [25], whereas, contrary to that opinion, positive samples were obtained around infected poultry farms during an outbreak; signifying a potential of the virus to get airborne and to spread by this route [56]. Much as this route is often underrated, a quantitative assessment of its possible contribution during the Dutch 2003 HPAI epidemic revealed that it could have made a substantial contribution to the between-farm spread of the virus (Chapter 2). In order to reduce disease spread by this route, existing control measures such as keeping all free range birds indoors during epidemics should be supplemented by measures to reduce dust emissions from poultry houses and the dispersal range of the emitted dust as well as installing air scrubbers on poultry houses.

Improving on-farm biosecurity practices: lessons from crisis organisation contacts

Factors such as absence of facilities, non-compliance with existing protocols and non-exhaustiveness of protocols reported in Chapter 4 negatively affect the effectiveness of biosecurity in controlling disease introduction and spread. The low per-contact probability of infection and the small number of secondary infections caused by

crisis organisation contacts (Chapter 3) demonstrates the effectiveness of their protocol in controlling virus transmission. Much as these contacts are most likely to occur with the most dangerous farms, i.e. those that are more likely to be already in their infectious stage, it was found (in Chapter 3) that their subsequent visits were less risky than those by the other traced contacts. On the basis of this finding, it is recommended that if possible, the daily farm biosecurity protocols be adjusted to 'mimic' those applied by the crisis organisation personnel. In anticipation of the difficulties in daily implementation, these improved protocols could be saved for crisis times or during dangerous farm visits such as restocking and thinning.

Combining genetic and epidemiological data when studying disease spread

On the one hand, contact-tracing (epidemiological) data obtained during epidemics can be used to guide the identification and quantification of individual contributions of potential transmission events [46] although some contacts may go unnoticed. On the other hand, genetic data obtained from the samples taken from the infected farms can be used to identify infection clusters [97] as well as to construct potential transmission trees as demonstrated in [100]. However, the infection clusters and the transmission trees identified will not give clues on the actual routes of transmission. In view of this, a combination of genetic and epidemiological data from epidemics of livestock diseases provides a better alternative to study between-farm transmission dynamics [101] and this approach was used in Chapter 3. The findings in Chapter 3 reveal that using genetic data alone is not enough to estimate transmission rates as therefore also information on contacts that fail to lead to transmission are needed. Genetic data can be used to check estimated transmission rates. In particular, it was found that the number of genetically matching farm pairs between which infection was likely to have occurred (based on the contact tracing data) would be as low as zero. Because of the possible 'competition' between potentially infectious contacts to a susceptible farm, improving the resolution on potential transmission events requires both genetic and epidemiological data to complement each other. Both forms of data are important for improved analyses of past epidemics and should be gathered whenever possible.

Instantaneous or delayed transmission: lessons from diffusion-based approaches

Spatio-temporal and other analyses of disease spread between locations involve making assumptions about the time lag between infectiousness of the source and the onset of its secondary infections. Often instantaneous transmission is assumed. However, delayed transmission has been shown (Chapter 5 and 6) to play an important role during epidemics and pathogen survival characteristics are known to determine how long the maximum delay can be. The possibility of delay in transmission makes that current recommended control strategies need to be reconsidered because farms can contribute to the infection hazard even when they are already stamped out.

Implications of the unknown, the overlooked and/or the untargeted pathways

The concept of neighbourhood transmission is a consequence of having infections that cannot be attributed to any known transmission route and is common during epidemics of livestock diseases [11,23,47]. Majority of the contributing contacts to the Dutch 2003 H7N7 HPAI epidemic were the 'unknown' contacts (Chapter 3). The 'unknown' transmission routes may constitute contacts or routes that are untargeted, untraced or simply untraceable. The list of potentially infectious contacts has been extended (Chapter 4) and attempts have been made to quantify the contribution of the untraceable contacts such as those due to the windborne route (Chapter 2). Improving management and control of livestock epidemics necessitates a better understanding of the day-to-day practices in the industry in order to regularly update the recommended control strategies during epidemics. This should be supplemented by an improvement in tracing efforts during epidemics.

Concluding remarks/recommendations/future perspectives

Quantitative modelling approaches are necessary as they can guide the development of the much wanted mechanistic insights into neighbourhood transmission. This insight is needed if control strategies are to be improved. The current guide on how to manage HPAI epidemics seems in-exhaustive. More HPAI transmission pathways have been hypothesized and their contribution quantified. The current biosecurity protocols should be updated to capture these. In addition to that, there is a laxity in implementing biosecurity on farms; this necessitates intervention by regulatory bodies to regularly sensitize stakeholders on the dangers of non-adherence to biosecurity as well as to regularly disseminate information about risk factors of disease introduction and spread. Control measures against HPAI spread by the windborne route should be incorporated in the current biosecurity and epidemic control protocols. In relation to data collection during epidemics, genetic and epidemiology data should both be gathered and used in studying the between-farm spread of livestock diseases. There is also need to revisit some of the existing assumptions about disease transmission between locations. Transmission of pathogens may not always be instantaneous since infectious material need time to travel between locations. The concept of delayed transmission should be incorporated in the analysis of neighbourhood transmission which also occurs in other settings like Intensive Care Units. This should change the way neighbourhood transmission is viewed.

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Summary

The poultry industry is a big contributor to various national economies worldwide and its productivity is heavily affected by the occurrences of epidemics of infectious diseases such as Highly Pathogenic Avian Influenza (HPAI) that are associated with big losses. The H7N7 HPAI epidemic that caused devastation in the Dutch poultry industry in 2003 is an example of such epidemics. During that epidemic, recommended control measures failed to control the epidemic. It is likely to have eventually been controlled by the depletion of susceptible farms. This strategy faced a lot of criticism from the general public thereby motivating the desire to design more 'ethically' acceptable intervention strategies such as vaccination. Such a vaccination strategy would be adopted as part of a wider set of control measures, with the other being biosecurity. However, successful implementation of these measures requires a better understanding of the mechanisms of between-farm spread of the virus. Moreover, in 2003, for about half of the outbreaks no indirect contact to a previous outbreak could be traced. For such outbreaks the cause of infection is often named "neighbourhood transmission".

In this thesis, an attempt was made to understand better how the between-farm transmission of HPAI during the Dutch 2003 epidemic continued despite the intervention strategies applied. The ultimate aim was to be able to improve these strategies such that their combination with a vaccination strategy could yield a sufficiently fast control of the epidemic. New data on possible HPAI transmission routes and mechanisms in the Netherlands was collected and modelling was used to analyse existing and new quantitative data. Subsequently, model extrapolations could be performed. Apart from the movement of animals, humans, vehicles or materials between farms, the contribution of other mechanisms (such as wind-borne transmission that cannot be controlled by regulations) to between-farm transmission of HPAI was quantified. It was hoped that more insight into the contributions of the different transmission mechanisms would enable a better formulation and/or prioritization of prevention and intervention measures directed against different routes.

There are several possible routes and mechanisms that could have facilitated the between-farm spread of HPAI that was observed during the Dutch 2003 H7N7 HPAI epidemic. One of the routes may be the wind-borne. The existence, in literature, of conflicting opinions about the significance of this route motivated the quest for quantitative information about its possible contribution. In Chapter 2 of this thesis, a mathematical model to determine the deposition pattern of wind-dispersed particles and to calculate the associated risk of infection with HPAI virus for a farm downwind of an infected farm as a function of the distance between the two farms was developed. This model is the first in the literature to mechanistically describe wind-borne transmission of HPAI. Findings from the analysis of this model showed that wind-borne transmission is a plausible contributor to between-farm transmission even beyond distances of a few kilometres. A comparison between the transmission risk pattern predicted by the model and the pattern observed during the Dutch 2003 epidemic reveals that the wind-borne route alone is insufficient to explain the observations. Rather, it could contribute to the spread in a direct wind-borne fashion as described by the model (potentially contributing up to 24% of all transmissions within 25 km) and additionally in a multi-stage process with wind-borne dispersion as one of the stages. The model developed can be adapted to other situations in which the spatial deposition pattern of wind-dispersed particles is an important determinant of risk, e.g. to human health.

Quantitative information such as the estimates of the probabilities of transmission via different types of indirect between-farm contacts during an epidemic provides insight into risks that need to be controlled as best as possible by prevention and intervention measures. In Chapter 3, using digitalized data on the traced and modelled contacts that occurred during the Dutch 2003 epidemic, per-contact probabilities of infection were estimated as well as the number of new cases attributed to particular routes. Findings from this analysis suggest that, among the traced contacts, egg transports had the highest probability of infection (i.e., 31%) and the between-farm contacts through visits of individuals involved in screening and other crisis-organisation activities (referred to as crisis organisation contacts) had the least (i.e., 0.1%). The findings further indicate that, compared to the other types of traced contacts, crisis organisation contacts contributed very little to transmission. This suggests that the biosecurity measures adopted by the teams involved perform well. Overall, the traced contacts were responsible for only approximately 7% of the total of outbreaks observed during the epidemic. A validation of the predicted number of new cases potentially caused by the different traced contact types against the sequencing data obtained from the samples collected from farms during the epidemic revealed that the two were consistent.

The separate analyses of wind-borne transmission and of the traced contacts data have shown that between approximately 50% and 80% of the observed transmission can neither be explained by traced contacts nor by wind-borne transmission. In order to learn more about possible risks in practice corresponding to the indirect contact types that are commonly hypothesized (and appearing in the Dutch 2003 epidemic tracing reports) as well any further possible indirect contact types (especially those that could underlie the untraced outbreaks), an interview study involving 60 stake holders was conducted (Chapter 4). The findings of this study guided the generation of better hypotheses about the underlying mechanisms. They can also facilitate the use of models to explore the hypothesized mechanisms. They provided information on the frequencies of different types of indirect contact and on farmer and firm perception of risks. In a qualitative risk assessment of the different contact risks identified, restocking, thinning, proximity to poultry farms and contacts accessing poultry houses were found to be the most important risks in relation to HPAI transmission. Farmers were found to have divergent opinions about the visitor- and neighbourhood- associated risks of HPAI transmission and there were no major differences found in infection risk between broiler and layer farms. Furthermore, the findings confirmed the expectation that not all biosecurity protocols are actually always complied with and provided insight into the actual violations. For example, the level of visitor adherence to available biosecurity protocols is not always good as evidenced by the result that on most of the farms, available showers were rarely or never used by visitors entering the animal houses. Most of the farms interviewed lacked a designated clean/dirty route. Many farms allowed visitors such as veterinarians to take personal belongings such as mobile phones into the animal house. These findings are relevant in the sense that, based on them, updates on contact tracing protocols to be used in a future epidemic can be made.

Neighbourhood transmission was further studied in this thesis by analysis of indirect transmission patterns observed in an experiment (Chapter 5), after which the findings were validated to the field data from the 2003 epidemic (Chapter 6). In the experiment, the possibility of delayed transmission, contrary to the common assumption of instantaneous transmission, manifested itself and this possibility was therefore also

investigated for the transmission in the field in 2003. A diffusion-based model –in which the displacement of an infectious agent through space is described by a step-by-step diffusion process– for transmission between locations was developed (Chapter 5) and applied to part of the Dutch 2003 H7N7 HPAI epidemic data (Chapter 6). Other than the realism of its underlying assumption on transmission, the diffusion-based model has the advantage that even without distinguishing transmission routes, outbreak data can be analyzed to gain insight into the between-farm transmission. This is facilitated by estimating an average daily displacement of the material. Findings from this study show that two-dimensional diffusion modelling is a promising approach to mechanistically describe untraceable indirect transmission in a parsimonious manner; with a small number of parameters that each can be feasibly estimated. This model proves successful in explaining experimentally observed different time-dependent patterns of indirect transmission for Campylobacter compared with E. Coli through the interplay between transport and decay of pathogens in the environment between infectious and susceptible hosts. In addition, a delayed transmission component, as predicted by the model, has been demonstrated in a dataset of antibiotic resistant bacterial infections in a hospital intensive care unit. By enhancing the quantitative understanding of the transmission events that can neither be explained by wind-borne transmission nor traced contacts, this new modelling approach contributes to the improvement sought in the overall understanding of between-farm transmission of HPAI.

The main conclusions of this thesis

This thesis has generated more insights into the mechanisms of between-farm transmission of HPAI. Unlike previous spatial analysis of the data of the Dutch 2003 H7N7 HPAI epidemic which used a statistical description of the total between-farm transmission (i.e. not distinguishing transmission routes) to quantify its distance dependence, here individual transmission routes were modelled. Generally, new insight has been obtained into the quantitative contributions of the different possible mechanisms of between-farm spread. This enables a better risk-based formulation and/or prioritization of prevention and intervention measures directed against different routes. Briefly, the following conclusions can be drawn from this thesis:

- The wind-borne route, although it may contribute to transmission, cannot (on its own) explain the observed pattern of the Dutch 2003 epidemic. The unexplained transmission may have been as a result of other mechanisms or a consequence of a multi-step process involving the wind-borne and other mechanisms.
- It was estimated that only 7% of the new cases can be attributed to a traced between-farm contacts. This indicates the existence of other possible transmission mechanisms that are untraced, untraceable and/or untargeted. Adjustment to the current tracing and (consequently) the biosecurity protocols can be recommended.
- The large share of unexplained transmission reaffirms the need to improve the conditions for successful contact tracing during future outbreaks, possibly by using virus sequencing to support the contact tracing.
- The biosecurity measures of the crisis organization teams seem sufficient to prevent the between-farm spread of HPAI. Therefore, the relevant authorities should adopt (some of) these measures for day-to-day use on poultry farms.

- Biosecurity breaches are common in day-to-day farm activities with the identified obstacles to proper biosecurity practices including; absence of facilities, non-adherence or inconsistent application, the non-exhaustive protocols.
- By applying a diffusion model to part of the Dutch 2003 HPAI epidemic data, it was found that this type of modelling is a scientifically promising approach to mechanistically describe indirect between-farm transmission in a parsimonious manner.

Nederlandse samenvatting

De pluimveesector levert een grote bijdrage aan verschillende nationale economieën in de wereld en haar productiviteit wordt ernstig geschaad wanneer epidemieën optreden van infectieziekten als hoog-pathogene aviaire influenza (HPAI), ook wel bekend als vogelpest, die grote verliezen met zich meebrengen. De H7N7 HPAI epidemie die in 2003 de Nederlandse pluimveesector zwaar trof is een voorbeeld van dergelijke epidemieën. Gedurende deze epidemie bleek dat het aanbevolen maatregelenpakket onvoldoende was om de epidemie onder controle te brengen. Dat deze uiteindelijk tot staan is gebracht is waarschijnlijk vooral vanwege de afname van het aantal overblijvende gevoelige bedrijven als gevolg van preventieve ruimingen. Het preventief ruimen van gezonde dieren kreeg veel kritiek in de publieke opinie, met als gevolg dat de wens bij beleidsmakers ontstond om maatschappelijk beter acceptabele interventiestrategieën te ontwikkelen zoals noodvaccinatie. Een dergelijke noodvaccinatiestrategie zou deel uitmaken van een breder pakket aan maatregelen, met daarin ook diverse bio-veiligheidsmaatregelen. Echter, om bio-veiligheidsmaatregelen met succes in te kunnen zetten is een beter begrip nodig van de mechanismen van verspreiding van het virus tussen pluimveebedrijven. Bovendien kon in 2003 voor ongeveer de helft van de uitbraakbedrijven geen contact met een eerder geïnfecteerd bedrijf worden getraceerd. De onbekende oorzaak van virus-insleep op deze bedrijven wordt vaak aangeduid met de term “buurttransmissie”.

In dit proefschrift werd een poging gedaan om beter te begrijpen hoe de verspreiding van HPAI tussen bedrijven in de Nederlandse epidemie van 2003 bleef doorgaan ondanks de genomen bestrijdingsmaatregelen. Het uiteindelijke doel was om in staat te zijn die maatregelen zodanig te verbeteren dat deze in combinatie met een noodvaccinatiestrategie tot een voldoende snelle controle van een toekomstige epidemie zouden leiden. Nieuwe gegevens over mogelijke routes en mechanismen van HPAI transmissie werden verzameld en wiskundige modellering werd gebruikt om bestaande en nieuwe kwantitatieve gegevens te analyseren. Vervolgens konden model-extrapolaties worden uitgevoerd. Naast bewegingen van dieren, mensen, voertuigen of materialen tussen bedrijven werd de bijdrage van andere mechanismen (zoals transmissie via wind die niet kan worden gecontroleerd door regelgeving) aan de tussen-bedrijfstransmissie van HPAI gekwantificeerd. Daarbij was de hoop dat meer inzicht in de bijdragen van de verschillende transmissiemechanismen een betere formulering en/of prioritering van preventie- en interventiemaatregelen gericht tegen de verschillende routes mogelijk zou maken.

Er zijn verschillende mogelijke onderliggende routes en mechanismen voor de tussen-bedrijfstransmissie van HPAI die optrad in de Nederlandse H7N7 epidemie in 2003. Een van de routes zou de transmissie via wind kunnen zijn. Het gebrek aan consensus in de wetenschappelijke literatuur over het belang van deze route vormde de aanleiding om naar kwantitatieve informatie daarover te zoeken. In Hoofdstuk 2 van dit proefschrift werd een wiskundig model ontwikkeld om het depositiepatroon van door wind verspreide deeltjes te bepalen alsmede het daarmee gepaard gaande risico van infectie met HPAI van een pluimveebedrijf benedenwinds van een geïnfecteerd bedrijf, als functie van de afstand tussen de twee bedrijven. Dit model is het eerste in de literatuur waarin transmissie via wind van HPAI mechanistisch wordt beschreven. Uitkomsten van de analyse van dit model lieten zien dat de transmissie via wind een plausibele contribuant aan tussen-bedrijfstransmissie vormt zelfs voor afstanden groter dan enkele kilometers. Een vergelijking tussen het transmissierisico-patroon zoals voorspeld door het model en het patroon geobserveerd tijdens de Nederlandse epidemie in 2003 toont aan dat de windverspreidingsroute alleen onvoldoende is om de

observaties te verklaren. In plaats daarvan zou de wind hebben kunnen bijdragen zowel in de vorm van directe windverspreiding zoals beschreven in het model (mogelijkerwijs verantwoordelijk voor een percentage van maximaal 24% procent van alle transmissie over afstanden tot 25 km) als ook in de vorm van een meerstaps-proces met dispersie door wind als een van de stappen. Het ontwikkelde model kan worden aangepast voor toepassing op andere vraagstellingen waarbij het ruimtelijke depositiepatroon van door wind verspreide deeltjes een belangrijke bepalende factor is, zoals bij bepaalde volksgezondheidsrisico's.

Kwantitatieve informatie zoals de kwantificering van de transmissiekansen via verschillende typen van tussen-bedrijfscontacten tijdens een epidemie geeft inzicht in welke contactrisico's zo goed mogelijk moeten worden beperkt middels preventie- en interventiemaatregelen. In Hoofdstuk 3 werden de infectiekansen per contact gekwantificeerd, alsmede de aantallen uitbraken toe te schrijven aan verschillende typen contact, gebruik makend van gedigitaliseerde gegevens over tijdens de epidemie in 2003 getraceerde contacten. De bevindingen van deze analyse suggereren dat, binnen de getraceerde contacten met bronbedrijven, het ophalen van eieren de grootste infectiekans met zich meebrengt (d.w.z. 31%) en de tussen-bedrijfscontacten door individuen betrokken bij screening en andere crisisorganisatie-activiteiten (kortweg betiteld als crisisorganisatie-contacten) de laagste kans (d.w.z. 0.1%). Bovendien geven de bevindingen aan dat, vergeleken met de andere typen getraceerde contacten, crisisorganisatie-contacten heel weinig aan transmissie bijdroegen. Dit suggereert dat de bio-veiligheidsmaatregelen die door de teams in acht werden genomen goed werkten. In totaal waren de getraceerde contacten verantwoordelijk voor slechts ongeveer 7% van het totaal aantal uitbraken in de epidemie. Een toetsing van het voorspelde aantal uitbraken veroorzaakt door de verschillende typen getraceerde contacten aan genetische (sequencing) gegevens voor de monsters genomen op die bedrijven tijdens de epidemie liet zien dat deze twee consistent waren.

De afzonderlijke analyses van de verspreiding via wind en van de gegevens van getraceerde contacten hebben laten zien dat tussen ongeveer 50% en 80% van de geobserveerde transmissie noch door getraceerde contacten noch door verspreiding via wind verklaard kan worden. Om meer te weten te komen over mogelijke risico's in de praktijk, zowel die gerelateerd aan de gewoonlijk veronderstelde typen indirecte contacten (die ook voorkomen in de traceringsrapporten tijdens de Nederlandse epidemie in 2003) als mogelijke verdere typen indirecte contacten (in het bijzonder die welke de oorzaak zouden kunnen zijn van uitbraken zonder getraceerd contact), werd een interviewstudie gehouden onder 60 betrokkenen bij de pluimveehouderij. De resultaten van deze studie gaven een leidraad voor het formuleren van betere hypothesen over onderliggende transmissiemechanismen. Ook maken deze het mogelijk om gebruik makend van modellen zulke hypotheses te verkennen. Ze gaven informatie over de frequentie waarmee de verschillende typen contacten voorkomen en over de risico-perceptie van pluimveehouders en pluimvee-gerelateerde bedrijven. In een kwalitatieve risicoanalyse van de verschillende contacten gevonden in de interviewstudie werden aanvoer van pluimvee, uitdunnen, nabijheid van andere pluimveebedrijven en personen die stallen betreden aangemerkt als de belangrijkste risico's met betrekking tot HPAI transmissie. Pluimveehouders bleken uiteenlopende opinies te hebben over de HPAI transmissierisico's verbonden met bezoekers en de

omgeving, en er warden geen grote verschillen gevonden in infectierisico's tussen vleeskuikenbedrijven en legbedrijven. Daarnaast bevestigden de uitkomsten de indruk dat niet alle bio-veiligheidsprotocollen altijd worden aangehouden, en gaven deze inzicht in de precieze protocolschendingen. Zo worden bijvoorbeeld de beschikbare protocollen voor bezoekers niet altijd goed aangehouden, zoals blijkt uit het feit dat op de meeste bedrijven de beschikbare douches zelden of nooit door bezoekers die de stallen betreden werden gebruikt. De meeste geïnterviewde bedrijven hadden geen gemarkeerde scheiding van "schone en vuile weg". Veel pluimveehouders stonden bezoekers zoals dierenartsen toe om persoonlijke eigendommen zoals mobiele telefoons de stal in mee te nemen. De bevindingen van deze studie zijn van belang als input voor het updaten van traceringsprotocollen voor gebruik bij toekomstige uitbraken.

Buurttransmissie werd in dit proefschrift verder bestudeerd door analyse van indirecte transmissie in een experiment (Hoofdstuk 5), waarna de bevindingen werden getoetst aan de veldgegevens uit 2003 (Hoofdstuk 6). In het experiment kwam de mogelijkheid van vertraagde transmissie, in tegenstelling tot de gewoonlijk veronderstelde onmiddellijke transmissie, aan het licht en deze mogelijkheid werd daarom ook onderzocht voor de transmissie in het veld in 2003. Een model gebaseerd op diffusie – waarin de verplaatsing van een infectieuze kiem wordt beschreven door de ruimte wordt beschreven door een stap-voor-stap diffusieproces – werd ontwikkeld voor de transmissie tussen (pluimvee)locaties (Hoofdstuk 5) en dit werd vervolgens toegepast op de gegevens van de Nederlandse H7N7 HPAI epidemie in 2003 (Hoofdstuk 6). Afgezien van de realistische beschrijving van het veronderstelde mechanisme onderliggend aan transmissie, heeft het diffusie-gebaseerde model het voordeel dat zonder verschillende individuele transmissieroutes te onderscheiden uitbraakgegevens kunnen worden geanalyseerd om inzicht te krijgen in de tussen-bedrijfstransmissie. Dit wordt mogelijk gemaakt door een gemiddelde dagelijkse verplaatsing van het materiaal te schatten. De bevindingen van deze studie laten zien dat tweedimensionale diffusiemodellering een veelbelovende aanpak is om niet-traceerbare indirecte transmissie op een "spaarzame" wijze te beschrijven: met een klein aantal parameters die elk redelijkerwijs gekwantificeerd kunnen worden. Deze modellering blijkt succesvol in het verklaren van experimenteel waargenomen tijdsafhankelijke patronen van indirecte transmissie voor *Campylobacter* en *E. Coli* en de verschillen daartussen, in termen van het samenspel tussen transport en verval van de kiemen in de omgeving tussen infectieuze en gevoelige gastheer. Daarnaast is een vertraagde component van transmissie, zoals voorspeld door het model, aangetoond in gegevens voor infecties met een bacterie met antibiotica-resistentie op een intensive-care afdeling van een ziekenhuis. Door het vergroten van ons kwantitatieve begrip van die gevallen van transmissie die noch door windverspreiding noch door getraceerde contacten kunnen worden verklaard, draagt deze nieuwe aanpak van modellering bij aan de gewenste verbetering van ons algehele begrip van de tussen-bedrijfstransmissie van HPAI.

De hoofdconclusies van dit proefschrift

Dit proefschrift heeft meer inzicht opgeleverd in de mechanismen van tussen-bedrijfstransmissie van HPAI. Anders dan eerdere ruimtelijke analyse van de gegevens van de Nederlandse H7N7 HPAI epidemie in 2003, die een statistische beschrijving gebruikte van de totale tussen-bedrijfstransmissie (d.w.z. geen onderscheid makend

tussen verschillende transmissieroutes) om de afstandsafhankelijkheid daarvan te kwantificeren, werden hier wel individuele transmissieroutes gemodelleerd. In het algemeen is nieuw inzicht verkregen in de kwantitatieve bijdragen van de verschillende mogelijke mechanismen van verspreiding tussen bedrijven. Dit maakt een betere risico-gebaseerde formulering en/of prioritering van preventie- en interventiemaatregelen gericht tegen verschillende verspreidingsroutes mogelijk. In het kort kunnen de volgende conclusies worden getrokken uit dit proefschrift:

- De route via wind, hoewel deze kan bijdragen aan transmissie, kan (alleen) het geobserveerde patroon van transmissie in de epidemie van 2003 niet verklaren. De onverklaarde transmissie kan het gevolg zijn geweest van andere mechanismen of van een meerstaps-proces waarin wind en andere mechanismen een rol spelen.
- Naar schatting slechts 7% van de uitbraken kan worden toegeschreven aan getraceerde tussen-bedrijfscontacten. Dit suggereert het bestaan van andere mogelijke transmissieroutes die niet getraceerd, niet-traceerbaar en/of niet nagegaan zijn. Daarom zouden de huidige traceringsprotocollen en (dus) de bio-veiligheidsprotocollen moeten worden bijgesteld om deze mechanismen daarin te verdisconteren.
- Het grote aandeel van onverklaarde transmissie bevestigt opnieuw de noodzaak om de voorwaarden te verbeteren voor succesvolle tracering bij toekomstige uitbraken, mogelijkerwijs door het gebruik van virus sequencing om de tracering te ondersteunen.
- De bio-veiligheidsmaatregelen genomen door de crisis-organisatieteams lijken voldoende om tussen-bedrijfstransmissie van HPAI door deze teams te voorkomen. Daarom zouden de relevante autoriteiten (sommige van) deze maatregelen moeten invoeren voor dagelijks gebruik op pluimveebedrijven.
- Schendingen van bio-veiligheid komen vaak voor bij de dagelijkse activiteiten op pluimveebedrijven, waarbij de volgende obstakels voor het realiseren van een goede bio-veiligheid optreden: afwezigheid van faciliteiten, het zich niet houden aan protocollen of inconsistente toepassing daarvan, en onvolledigheid van protocollen.
- Uit het toepassen van een diffusiemodel op een deel van de gegevens van de Nederlandse HPAI epidemie in 2003 blijkt dat deze modellering een wetenschappelijk veelbelovende aanpak is om indirecte transmissie tussen bedrijven op een “spaarzame” manier mechanistisch te beschrijven.

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First and foremost, I would like to express my sincere gratitude to my PhD supervisors and mentors; Dr. Thomas Hagenaars and Prof. Mart de Jong. If it were not for your guidance and a great level of patience, this project may have not been this fruitful. Your supervision style has left a permanent impression in me and I very much envy it. You have helped me realise my potential as researcher, a process that began with trusting and appointing me to the position and went on through the daily re-assurances. I gained a lot from the numerous discussion-turned-into-lecture sessions. I should also point out the fact that I enjoyed the lifts to Wageningen with Mart as they were filled with discussions. I also benefited a lot from Thomas' organisation skills; it is one of the key reasons for having had a smooth running of this project..

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Curriculum vitae

Amos Ssematimba was born on 4 June 1979 in Mpigi district, Uganda. In 2002, he graduated with a bachelor of science with education (mathematics and physics) at Mbarara University of Science and Technology. In the same year, he joined Gulu University as a teaching assistant in the department of mathematics and has since been promoted to the position of lecturer and head of department at the same institute. He joined Makerere University in 2003 to pursue a master of science degree in mathematics which he completed in 2006 by producing a dissertations about mathematical modelling of the dynamics of tuberculosis. In September 2008, he got the opportunity to work as PhD student/research assistant (AIO) at the Quantitative Veterinary Epidemiology group of Wageningen University and his work station was at the Central Veterinary Institute part of Wageningen UR in Lelystad. The project involved using mathematical modelling techniques to understand the between-farm spread of avian influenza. From 3rd September 2012, Amos was appointed as a post-doctoral scientist at the International Livestock Research Institute based in Nairobi, Kenya. The post-doc involves the use of mathematical models to guide the generation of better control measures against contagious bovine pleuropneumonia. He intends to re-join Gulu University at the end of the post-doc to continue doing what he loves most; lecturing.

List of publications

Refereed scientific journals

Ssematimba, A., Hagenaars, T.J., de Wit, J.J., Ruiterkamp, F., Fabri,T.H., Stegeman, J.A, de Jong, M.C.M. (2012), Avian Influenza transmission risks: analysis of biosecurity measures and contact structure in Dutch poultry farming. Preventive Veterinary Medicine.doi:10.1016/j.prevetmed.2012.09.001

Ssematimba, A., Elbers, A.R.W., Hagenaars, T.J., de Jong, M.C.M. (2012), Estimating the per-contact probability of infection by highly pathogenic avian influenza during the 2003 epidemic in The Netherlands. PLoS ONE (2012), 7(7): e40929 doi:10.1371/journal.pone.0040929

Ssematimba, A., Hagenaars TJ, de Jong MCM (2012) Modelling the Wind-Borne Spread of Highly Pathogenic Avian Influenza Virus between Farms. PLoS ONE 7(2): e31114. doi:10.1371/journal.pone.0031114

Mugisha, J.Y.T, **Ssematimba, A.,** L.S. Luboobi and Ddumba, H. (2008), Modelling the critical characteristic area for the control of tuberculosis in densely populated communities, The Journal of Mathematical Control Science and Application, **2(2)**: 231-242

Ssematimba, A., Mugisha, J.Y.T. and Luboobi, L.S. (2005), Mathematical Models for the Dynamics of Tuberculosis in Density-Dependent populations: The case of Internally Displaced Peoples' Camps (IDPCs) in Uganda, Journal of Mathematics and Statistics, **1(3):** 217-224

Manuscripts in preparation

van Bunnik BAD, **Ssematimba A**, Hagenaars TJ, Nodelijk G, Haverkate, MR, Bootsma, MCJ, Bonten, MJM, de Jong MCM (2012), Small distances can keep bacteria at bay for days.

Ssematimba, A., Hagenaars TJ, Gert-Jan Boender, van Bunnik BAD , de Jong MCM (2012), Mechanistic modelling of highly pathogenic avian influenza transmission risk: the role of delayed transmission

Conference proceedings and abstracts

Ssematimba, A., Hagenaars, T.J., de Jong, M.C.M. Using mathematical models to determine the spatial extent of Virus/dust dispersal by wind. WIAS Science day, 12 March 2009, Wageningen. Poster.

Ssematimba, A., Hagenaars, T.J., de Jong, M.C.M. Revising the cost of controlling Avian Influenza epidemics: Using mathematical models to explore the possibility of airborne spread in the Dutch 2003 epidemic. International Workshop in Mathematical and Economic Epidemiology August 3 – 5, 2009, Makerere University, Kampala, Uganda. Poster.

Ssematimba, A., Hagenaars, T.J., de Jong, M.C.M. Assessing the role of dust dispersion in neighbourhood transmission of HPAI based on the Dutch 2003 H7N7 epidemic data. ISVEE Conference XII, 2009, Durban, South Africa, 10-14 August 2009. Oral.

Ssematimba, A., Hagenaars, T.J., de Jong, M.C.M. Modelling airborne spread of avian influenza between flocks, Proceedings of the Symposium on Epidemiology and Welfare, Dutch Society for Veterinary Epidemiology and Economics, Deventer, The Netherlands, 26 November 2009, p. 18-20. Oral.

Ssematimba, A., Hagenaars, T.J., de Jong, M.C.M. Highly Pathogenic Avian Influenza transmission risks: analysis of biosecurity measures and contact structures in Dutch poultry farming. Annual meeting: Society of Veterinary Epidemiology and Preventive Medicine March 24th – 26th, 2010, Nantes, France. Poster.

Ssematimba, A., Hagenaars, T.J., de Jong, M.C.M. Modelling the windborne spread of Highly Pathogenic Avian Influenza virus between farms. WIAS Science day, 3rd February 2011, Wageningen. Oral.

Ssematimba, A., Elbers, A.R.W., Hagenaars, T.J., de Jong, M.C.M. Estimating the per-contact probability of infection by highly pathogenic avian influenza during the 2003 epidemic in The Netherlands. Annual meeting: Society of Veterinary Epidemiology and Preventive Medicine March 23rd – 25th, 2011, Leipzig, Germany. Poster.

Ssematimba, A., Elbers, A.R.W., Hagenaars, T.J., de Jong, M.C.M. Estimating the per-contact probability of infection by highly pathogenic avian influenza during the 2003 epidemic in The Netherlands. The fourth Influenza conference, September 7th – 9th, 2011, Oxford, United Kingdom. Oral.

Ssematimba, A., Hagenaars, T.J., de Jong, M.C.M. The role of airborne transmission in the spatial spread of highly pathogenic avian influenza. The 4th Cees Wensing Lecture, Auditorium, Edelhertweg 15, Lelystad, the Nerthelands, 24th November 2011. Oral.

Ssematimba, A., Elbers, A.R.W., Hagenaars, T.J., de Jong, M.C.M. Assessing the contributions of the various between farm contacts to the spread of highly pathogenic avian influenza (H7N7) virus during the 2003 epidemic in the Netherlands. ISVEE Conference XIII, 2012, Maastricht, The Netherlands, 20-24 August 2012. Oral.

van Bunnik, B.A.D., **Ssematimba, A.** Hagenaars, T.J. , Nodelijk, G., Haverkate, M.R., Bootsma, M.C.J., Bonten, M.J.M., de Jong, M.C.M. Small distances can keep bacteria at bar for days. ISVEE Conference XIII, 2012, Maastricht, The Netherlands, 20-24 August 2012. Oral.

Training and Supervision Plan

Training and Supervision Plan

Wageningen Institute of Animal Sciences (WIAS)

Education and Training	**Year**
The Basic Package (3 credits)	
WIAS Introduction Course	2009
WIAS Course on philosophy of science and ethics	2010
Scientific Exposure (23 credits)	
International conferences	
International Symposium on Veterinary Epidemiology and Economics (ISVEE 12), Durban, South Africa, 10th-14th August	2009
International Symposium on Veterinary Epidemiology and Economics (ISVEE 13), Maastricht, The Netherlands, 20th-24th August	2012
Annual meeting of the Society for Veterinary Epidemiology and Preventive Medicine (SVEPM), Nantes, France: 24th-26th March	2010
Annual meeting of the Society for Veterinary Epidemiology and Preventive Medicine (SVEPM), Leipzig, Germany, 23rd -25th March	2011
The 5th Epizone Annual meeting, Arnhem, The Netherlands, 11th - 14th April	2011
The 6th Epizone Annual meeting, Brighton, United Kingdom, 12th-14th June	2012
The 4th Oxford avian influenza conference, Oxford, United Kingdom, 7th-10th September	2011
Seminars and workshops	
Workshop on Indirect Transmission, Ambiance Houtrust, Amersfoort, The Netherlands, 25th September	2008
Workshop on Analysis and Numerics of Population dynamics and Epidemics models, Udine, Italy, 15th-17th December	2008
Annual meeting of the Dutch Society of Theoretical Biology(NVTB), Schoorl, The Netherlands, 7th-8th May	2009
WIAS Science Day, Wageningen, The Netherlands, 12th March; 28th January; 3rd February	2009+ 2010 +2011
The UCID symposium on infection dynamics, Spoorweg museum, Utrecht, The Netherlands, 9th -11th March	2011
Presentations	
WIAS Science Day, Wageningen, The Netherlands, 12th March, Poster	2009
Annual meeting of the SVEPM 2010, Nantes, France, 24-26 March, Poster	2010
Annual meeting of the SVEPM 2011, Leipzig, Germany, 23rd -25th March,Poster	2011

Mathematical epidemiology international workshop, Makerere University, Kampala, Uganda, 3rd-5th August, Poster	2009
International Symposium on Veterinary Epidemiology and Economics (ISVEE 12), Durban, South Africa, 10th-14th August, Oral	2009
Dutch Society for Veterinary Epidemiology and Economics (VEEC), Deventer, The Netherlands, 26th November, Oral	2009
FES plenary meeting, Auditorium, Edelhertweg 15, Lelystad, The Netherlands, October, Oral	2009
WIAS Science Day, Wageningen, The Netherlands, 3rd February, Oral	2011
The 4th Oxford avian influenza conference, Oxford, United Kingdom 7th-10th September, Oral	2011
The 4th Cees Wensing Lecture, Auditorium, Edelhertweg 15, Lelystad, The Netherlands, 24th November,Oral	2011
The 6th Epizone Annual meeting, Brighton, United Kingdom, 12th-14th June, Oral	2012
International Symposium on Veterinary Epidemiology and Economics (ISVEE 13), Maastricht, The Netherlands, 20th-24th August, Oral	2012
In-Depth Studies (25 credits)	
Disciplinary and interdisciplinary courses	
Summer School on Mathematical Ecology and Evolution, Turku, Finland, 22nd -29th August	2010
Mathematical epidemiology of infectious diseases, Utrecht University, Utrecht, The Netherlands, January-June	2009
Design and Analysis of Transmission Experiments, Wageningen University, Wageningen, The Netherlands, 2nd-6th November	2009
Advanced statistics courses	
Modern statistics for the life sciences, Wageningen University, Wageningen, The Netherlands, 4th January-23rd February	2010
PhD students' discussion groups	
Reading group on mathematical modelling of infectious disease	2008-2009
Monthly scientific meeting QVERA/QVE	2008-2012
Journal club (twice a month)	2009-2010
Professional Skills Support Courses (6 credits)	
Writing for academic publication	2010
Project- and Time Management	2010
Writing the PhD thesis and its propositions	2011
High-Impact Writing in Science	2012
Research Skills Training (2 credits)	
Preparing own PhD research proposal	2009
Didactic Skills Training (1 credit)	
Lecturing	

MTEC Course	2009
Education and Training Total	**60**

* one ECTS credit equals a study load of approximately 28 hours

Colophon

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