Part A – Applicant

A.1 Applicant

Name student (initials, first name, last name, student number):	F.L.(Floor) Veldhuis, 5835755
Affiliation (university/institute + department):	Utrecht University / Veterinary medicine department of Farm Animal Health
Name first examiner:	Dr. F.C. (Francisca) Velkers
Affiliation (university/institute + department):	Utrecht University/Veterinary medicine department of Farm Animal Health
Name second examiner:	Prof. dr. ir. Lidwien A.M. Smit
Affiliation (university/institute + department):	Utrecht University/ Veterinary medicine department of Risk Assessment Sciences

Part B – Scientific proposal

B.1 BASIC DETAILS

B.1.1 Title

Assessing the particle types involved in the airborne transmission of avian influenza virus and estimating the risk with optimized mathematical models.

B.1.2 Abstract

Since October 2021 Europe is experiencing the largest highly pathogenic avian influenza virus (HPAIV) epidemic so far, causing enormous losses of wild birds and poultry. Despite strict biosecurity measures, farms keep getting infected. Airborne transmission at the wild-domestic interface and between farms is one the routes that is thought to play a role in these introductions. Poultry farms emit large amounts of particulate matter (PM) into the environment that can serve as vectors for (HP)AIV (RNA). Moreover, airborne spread may impose a risk for public health, as some variants of the virus are zoonotic. Current risk assessment models predict risks based on particles with standard aerodynamic diameters, but behaviour of feather (material) and other organic material, and the viability of HPAIV associated with different particles is largely unknown. The aim of this project is to gain insight into the behaviour of and distribution of infectious virus over different particle types and to improve risk assessment models for airborne transmission. Model input parameters will be collected from the literature, laboratory, and field experiments. Current mathematical models will be adapted, parameterized with the collected data, and validated. This model will serve as a tool for policymakers and will allow for improved risk-based control measures to reduce opportunities of HPAIV to be transmitted from wild birds to poultry and vice versa, between poultry farms and to mammalian hosts, including humans.

B.1.3 Layman's summary

Sinds oktober 2021 ondervindt Europa een grote vogelgriepuitbraak met een hoog pathogene variant van het aviaire influenzavirus (HPAIV). Tijdens deze uitbraak zijn er al enorme aantallen wilde vogels en pluimvee doodgegaan. De manier waarop het virus zich verspreidt is veranderd. Waar vroeger wilde vogels bekend stonden om het verspreiden van de laag pathogene variant van het virus (LPAI), kunnen ze de huidige varianten van het HPAIV tijdens de vogeltrek over grote afstanden verspreiden wat kan leiden tot uitbraken bij commercieel gehouden pluimvee. Ondanks strikte hygiënemaatregelen op pluimveebedrijven, en ophok- en afschermplichten, is gebleken dat het virus toch de stal kan binnenkomen. Maatregelen om uitbraken te voorkomen en het snel ruimen van besmette bedrijven blijken niet meer voldoende effectief én door de grote aantallen pluimvee dat moet worden gedood, is het geen duurzame strategie meer. Het virus kan zich op verschillende manieren verspreiden, één van de manieren is via de lucht. Deze route is niet alleen van belang voor de verspreiding van virus van wilde vogels naar pluimveebedrijven en tussen pluimveebedrijven, maar ook voor de volksgezondheid. De vogelgriepvariant die we nu in Nederland hebben is niet heel gevaarlijk voor mensen, maar als het virus muteert of er komt een andere gevaarlijke variant mee met de trekvogels, dan is het belangrijk om te weten of mensen die in de buurt van een besmette stal wonen gevaar lopen. Over de transmissie via de lucht is nog veel onbekend. Bekend is dat kippenstallen erg veel kleine stofdeeltjes uitstoten, welke voornamelijk bestaan uit mestdeeltjes en veertjes. We weten dat het virus op deze deeltjes zit en zo meegenomen kan worden door de wind. Wat we nog niet goed weten is hoe het infectieuze virus verdeling verdeeld is over deze deeltjes en hoe deze deeltjes zich bewegen in de lucht. Wiskundige modellen die het risico van transmissie via de lucht inschatten doen dit door te rekenen met deeltjes die rond van vorm zijn, wat voor het merendeel van de deeltjes uit een stal niet geldt. Kennis over de verdeling van besmettelijk virus over de verschillende soorten stofdeeltjes en het gedrag van deze deeltje in de lucht is essentieel voor een goede inschatting van de risico's. Tijdens dit project willen we de ontbrekende kennis vergaren uit zowel literatuur, laboratorium- en veldexperimenten. Bestaande wiskundige modellen worden aangepast en met de gevonden data zal het effect van verschillende scenario's worden nagebootst door middel van simulaties. Het model maakt het mogelijk voor beleidsmakers om betere maatregelen te ontwerpen die meer effectief en duurzaam zijn. Door de kans op verspreiding van vogelgriepvirus te verminderen wordt er minder pluimvee ziek en is er minder kans dat het virus een gevaar vormt voor de volksgezondheid.

B.1.4 Keywords

Avian influenza virus; airborne transmission; between-farm transmission; particle size; mathematical modelling

B.2 SCIENTIFIC PROPOSAL

B.2.1 Research topic

Avian influenza virus (AIV) is a highly contagious influenza A virus belonging to the Orthomyxoviridae family and is an enveloped virus with a segmented negative stranded RNA genome. Several subtypes of AIV are defined based on two surface proteins: hemagglutinin (HA, H1-H16)) and neuraminidase (NA, N1-N9) (Spackman, 2008). AIVs are classified as low pathogenic avian influenza viruses (LPAIVs) or highly pathogenic avian influenza viruses (HPAIVs). All subtypes of HA can be of low pathogenicity, but HPAIV variants belong to the subtypes H5Nx and H7Nx (Swayne & Suarez, 2000). The virus is highly contagious for poultry, and in case of highly pathogenic subtype, causes serious disease with failure of multiple internal organs. Mortality can go up to 90 – 100% (Alexander, 2000).

Airborne transmission of AIV, one of the modes of transmission identified for the spread between farms (Jonges et al., 2015; Scoizec et al., 2018; Ypma et al., 2013; Zhao et al., 2019) is not yet well understood. The overall aim of this project is to fill in the knowledge gap about infectious virus distribution over different particle types and sizes, and the behaviour of these particles via the air. This information is essential in designing sustainable and effective control measures based on proper risk assessment tools, which will allow for improved control and hence reduction of opportunities of HPAIV to be transmitted from wild birds to poultry and vice versa, as well as to mammalian hosts, including humans. The latter is important if a zoonotic variant is circulating in the Netherlands. In this way, a good estimate of the risk can be made for residents and occupants and effective measures can be taken to hopefully prevent human disease and potentially even a pandemic. In de remainder of this section, we will describe which knowledge is lacking and needed, followed by the formulation of our research questions.

Epidemiology

Until 2002, the epidemiology was different from the current situation. LPAIV circulated in wild aquatic birds and new variants out of Siberia and Asia were seasonally introduced by migratory aquatic birds. Infections of poultry farms occasionally resulted in mutation to HPAIV that could spread further between farms. Since 2002, HPAIV is also circulating in wild aquatic birds after spill over from poultry to wild birds and can be spread intercontinentally by migratory birds (Bodewes & Kuiken, 2018). Furthermore, HPAIV recently has been found to remain circulating in resident birds in the Netherlands, shifting the seasonally epidemic situation linked to bird migration periods to a more endemic situation year-round (Germeraad et al., 2022). Since the 26th of October 2021, HPAIV H5Nx is causing outbreaks in 37 European countries, responsible for 47.7 million birds being culled (Adlhoch et al., 2022). Until now (16th of October 2022) 91 locations with commercial or small-scale (backyard) poultry have been infected in the Netherlands (Ministerie van Algemene Zaken, 2022a).

Control

The current control measures follow the stamping out principle, together with far-reaching preventive measures such as the preventive culling of nearby farms, confinement obligation of poultry (sometimes nationwide), and other restrictions, which are all of major impact for the poultry sector (Ministerie van Algemene Zaken, 2022b). In accordance with these control measures, over 4 million infected birds and over 9 thousands of potentially infected birds have been culled since (Ministerie van Algemene Zaken, 2022a). These control measures date from the before 2002. Back then, these measures of stamping out were effective. However, in the current “endemic” situation, infections are often introduced independently by wild birds. Therefore, the stamping out policy is not as effective as before and is most of all not sustainable. There is a need for more effective or novel control measures, e.g., preventive vaccination or more risk-based interventions aimed at the most likely routes of introduction of AIV and transmission between poultry farms, with special focus on high-risk farms. This may reduce the duration of a HPAIV epidemic and the associated control measures, as well as the number of affected farms and culled poultry.

One Health: the risk for human transmission

The current HPAIV H5N1 variant that is circulating in the Netherlands is of low risk for transmission to humans (Germeraad et al., 2022). For the virus to spread to and between humans it needs to undergo several adaptations to the mammalian body, including those associated with receptor binding and the lower body temperature (Gambotto et al., 2008). Receptors for AIV are present in the human airway, which indicates that these viruses can be transmitted from birds to humans. However, these receptors are located in the lower respiratory tract, making binding and efficient replication limited and therefore human-human transmission inefficient (Shinya et al., 2006). Nevertheless, in the Netherlands, 3 foxes have died of an infection with HPAIV H5N1 (Bordes et al., 2022), alerting us that adaptions of AIV to the mammalian body are possible and that we need to be prepared for a future with zoonotic variants.

Next to the threat of adaptation of the currently circulating virus, in Asia zoonotic variants are circulating and have caused infections in humans, with case fatalities up to 42% (Adlhoch et al., 2022). We cannot rule out the possibility of these variants being introduced into the Netherlands by migratory birds. To be prepared for these situations, knowledge about the modes of airborne transmission of AIV is highly needed.

For the zoonotic risk, particle deposition in the (human) respiratory tract also plays a role. The deposition of particles is dependent on the size, weight/particle inertia, shape, and the type of airflow in the respiratory tract. As a result, small particles <1µm can be deposited in the alveoli (deep respiratory tract) and particles > 1µm in the upper airways, see **figure 1** (Wang et al., 2021). The particles emitted by poultry farms are for 99% < 1µm, of which the majority originates from faeces and a smaller amount from feathers (Aarnink et al., 2011), which are prone to deposit into the lower respiratory tract. However, the infectious virus particles emitted by poultry farms have found to be related to particles >1µm (Alonso et al., 2015). Such particles sizes are less prone to deposit into the lower respiratory tract. In this project, particle types and sizes will be studied and linked to infectiousness to contribute to a better risk assessment.

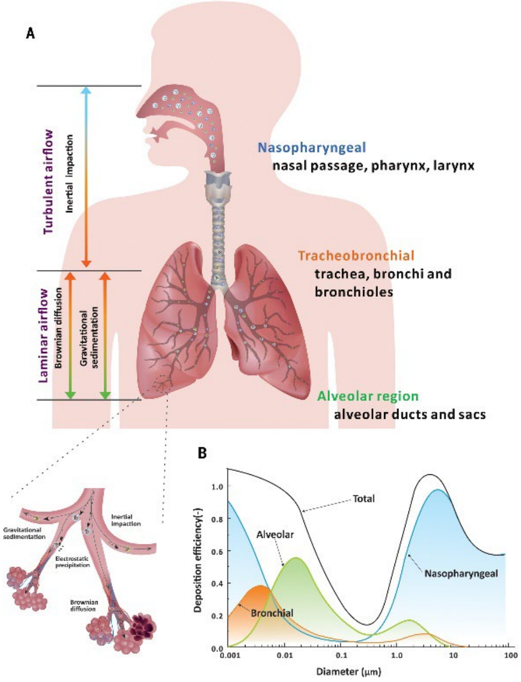


Figure 1: Size dependent aerosol deposition mechanisms to site in the respiratory tract. (A) The human respiratory tract and the deposition mechanisms, i.e., inertia impaction, for larger particles and gravitational sedimentation and Brownian diffusion for small particles. (B) Illustration of the efficiency of particle deposition at various locations. Figure taken from Wang et al. (2021).

Airborne transmission

Despite efforts of farmers to secure a high level of biosecurity, farms keep getting infected with AIV. There are many ways how the virus can be introduced. This mostly occurs via vectors and fomites, such as wild birds, rodents, farmers, vehicles, equipment etc. Farmers also fear airborne transmission, i.e. virus transmission from infected farms via the wind. Poultry farms are known to have a high emission of particulate matter. Most of these particles originate from faeces and feathers (Aarnink et al., 2011). Infected birds shed high levels of virus via the faeces (Germeraad et al., 2019). These virus particles can become airborne either by themselves or with the use of particulate matter as a vehicle, after which they can travel to neighbouring farms downwind and be introduced via the ventilation inlet (Ypma et al., 2013). In the current epidemic, genetic analysis done by Wageningen Bioveterinary Research (WBVR) has shown three clusters of infected poultry farms, where the genetic variation was very small between farms in the same cluster. This may be explained by direct transmission between these farms, however introduction via the same (wild bird) source cannot be ruled out as well. These farms were located close to each other and had no indications of high-risk contacts, suggesting that other introduction routes, such as airborne transmission, may have played a role (Germeraad et al., 2022).

Modelling studies

Studies have used epidemiological data to build mathematical models to calculate the probability of between-farm airborne transmission. Zhao et al. (2019) investigated the outbreaks of H5N2 in 2015 in Iowa, United States of America. They used the HYSPLIT model (more about this model in the method section) that used the air movement trajectories and the virus concentrations to assess the risk of airborne transmission in 77 cases of HPAIV. The virus concentration data was based on default and ceiling parameters adapted from literature. The question is how accurate these parameters are, because little is known about the infectivity of the virus bound to the particulate matter. The results showed that the majority of the farms might have been exposed to airborne virus, but the concentrations never exceeded the infectious dose. They argue that prolonged exposure to this airborne virus could have increased the risk. The model’s worst-case scenarios showed that 33 of the 77 farms were at medium to high risk of airborne exposure.

For the 2003 H7N7 outbreak in the Netherlands, Ypma et al. (2013) combined genetic and epidemiological data. They found that between-farm transmissions are more often in the direction of the wind than can be explained by chance and coordinates of poultry farms. Moreover, statistical analyses showed that the HPAIV spread was correlated with the wind direction and the date of infection. It was estimated that 18% of the infections were related to airborne transmission.

The particulate matter that is emitted into the air by poultry farms was captured up to 60m downwind by Jonges et al. (2015). Air samples were taken from five farms (1 chicken, 1 swan, and 3 turkey farms) infected with LPAIV and one control farm: inside, next to the air exhaust and further downwind. Virus RNA and endotoxins were detected, and emission rates were determined. For one of the farms, emission of 3x10^9 virus copies per hour was estimated, which indicates that many particles could be emitted into the environment and spread via the wind. Using atmospheric dispersion modelling (ADM) of particulate matter, areas at high risk for exposure could be identified. Questions arising from this research are if the virus particles remain infectious when spread by dust/particulate matter and what particle sizes the virus is associated with.

A different situation to the poultry farms we know in the Netherlands, but indicative for the risk of airborne spread of AIV, is described by Wei et al. (2018). These authors investigated the risk of downwind spread of LPAIV on live poultry markets (LPMs) in China. The sampling sites were chosen based on the predicted spread pattern of tracer gas for the weather conditions at that specific time. Up to 100m distance, concentration of 2.6x10^4 viral RNA copies per m3 were found. Computational Fluid Dynamics (CFD) modelling was used to include the presence of complex buildings. They found a combined effect of wind direction and surrounding buildings for the airborne transmission. With the daily poultry trade at those markets, it was suggested that windborne spread could expose the residents to the risk of infection via indirect contact.

Field experiments

At farm level, Scoizec et al. (2018) performed experiments to measure viral RNA. Samples were taken at duck farms (3) and chicken farms (2) inside, at the external exhaust and downwind. A decreasing trend in RNA concentration from external exhaust fans to downwind measure points was found, ascribed to dilution of the virus as a function of distance from the source. The findings were in line with the hypothesis of airborne transmission. One of the flocks was sampled during culling and viral RNA was detected inside the farm and 110m downwind, suggesting a potential risk of exposure to occupants nearby.

Particle size discrimination

In the studies above, it was not discussed how sizes and types of particles affect the viability of the virus. In the study of Alonso et al. (2017) particle size and concentration of viral RNA was measured with the use of two sampling devices. The Andersen Cascade Impactor (ACI) and Tish Cascade Impactor (TCI), which were both able to discriminate the particles by size. Sampling was performed inside and outside poultry farms (3 turkey, 1 layer flock). The samplers outside were located near- and at 5m distance to the air exhaust. The ACI sampler measured a higher amount of viral RNA than the TCI sampler for both inside and outside sampling, indicating a difference in performance. Higher concentrations of virus were associated with larger particles > 1um. For HPAIV (H5N2) a bimodal distribution in particle diameter was found, which could suggest multiple transmission mechanisms or transmission under varying environmental conditions. Lacking in this study is data on the viability of this virus at this particle size, which has been shown to be particle size dependent for other viruses, including swine influenza (Alonso et al., 2015).

Filaire et al. (2022) collected environmental samples (air, dust wipes, swaps) at 63 poultry farms. The air samples were taken at 19 of the 63 poultry farms with the use of two types of samplers: the Coriolis compact and the NIOSH BC. The samplers were able to discriminate particles by size. Infectiousness of the samples was tested by inoculating the virus in embryonated eggs and this was done for air samples taken from 5 poultry farms. Ten samples were tested (for three particle size fractions). Out of the ten tested samples, three were positive. High RNA load and infectious virus was associated with the largest particle size (>1um), suggesting that dust could be the major vehicle for the spread of AIV. The sample size of ten for virus inoculations was small and more of these tests should be done to statistically prove a relation between the particle size and viable virus.

Sampling methods

The methods of sampling not only influence the number of particles collected and the size distribution of these particles in the air (Alonso et al., 2017; Raynor et al., 2021), but the recovery of viable virus is influenced by the chosen methods as well. Raynor et al. (2021) compared various samplers, most of them of were impingers and cyclones. The total RNA and infectious virus were analysed for the different particle sizes. Samplers with a high flow have a low(er) detection limit and were able to detect RNA when present in low concentration. These samplers clearly were not favourable for the recovery of viable virus, because sampling processes like consolidation or evaporation of collection medium might damage the virus. Therefore, a two-sampler approach was recommended: the high flow for sampling virus with low concentrations and the low flow sampler to obtain infectious virus. Another interesting finding was that the samplers showed differences in the size distribution of the particles, which should be similar if the samplers would have performed equally well. This means that the choice of sampler may influence the outcome, which will be addressed in our pilot experiments.

Current knowledge and knowledge gaps

Although literature describes the spread of RNA downwind of poultry farms, the distribution of infectious particles over particle types is yet unclear. What is known are the types of particles that are emitted by poultry farms: most particles emitted by farms are <1µm, with the main fraction of particles <10µm originating form faeces, and the second contributor being feather materials (Aarnink et al., 2011). The rates of emission per animal for the size fractions of particulate matter <10µm

(PM10) and <2,5µm (PM2,5) ranged from 2.2 to 12.0mg per hour and 0.11 to 2.41mg per hour, respectively (Winkel et al., 2015). However, information about the particle types to which AIV is bound is lacking. Current models predict risks based on particles with standard aerodynamic diameters and do not consider different shapes. The behaviour in the air of feathers, feather material and other organic material is not yet well studied. It is of great importance to know how the infectious virus is distributed over the particle types and sizes and how these particles behave in the air.

Aim of the project

The overall aim of this project is to fill the knowledge gap about infectious virus distribution over the particle types and sizes, and the behaviour of these particles in the air. To fill in these knowledge gaps, we ask ourselves the following research question: What particle types play a role in the airborne transmission of infectious avian influenza virus between poultry (farms)? To investigate this, we have set several sub-goals. In a literature study we want to provide information about the particles emitted by farms, virus stability under different conditions, the devices with which we will sample the air and what kind of mathematical models are available to predict the spread of infectious virus particles. The best fitting model for our purpose will be fine-tuned, and missing parameters will be collected in lab and field experiments. The end product will be a model that serves as a risk-based model for science, government, and policymakers. In the approach, work packages are described, and the associated sub questions are stated.

B.2.2 Approach

The research project will be divided into four work packages: 1 Literature study; 2 Mathematical model selection/design; 3 Experiments to generate model input data; and 4 Model simulations and sensitivity analysis.

Work package 1: Literature study

The main goal is for the PhD student to get familiar with the subject, to review the literature that has been published between writing this proposal and the start of the project, and to define the relevant focus for his experiments, avoiding duplication.

The design: the following questions will be addressed during the literature study:

- What particles are transmitted into the air by poultry farms? This information is partly available in the literature, but the link between particle type and viability of the virus has not been made. Assessing the information about particle types out of the literature will help us to select the methods for our pilot study and the mathematical models for the risk assessment.
- What is the dispersion pattern of these particles? Information about how these particles behave in the air is needed for mathematical model selection and designing of (field) experiments.
- How viable is AIV in the outdoor environment in the different matrices? Virus decay under outdoor circumstances is needed for model predictions. The viability of AIV in/on particles will be investigated. For this, the literature will be studied for article/droplet/aerosol composition/structure and the effect on viability. Virus survival for instance is influenced by the presence of preen oil (Karunakaran et al., 2019) and will be considered in our project.
- What sample device strategies are available for the collection of various particle sizes and viable virus? This information is needed to select the best sampling strategy possible. An overview of the devices available will be given.
- What mathematical models are available in air pollution and disease modelling?
- What mathematical model (elements) from other disciplines can be used in modelling of the airborne spread of AIV? During the COVID-19 pandemic, several models have been developed, that could be of use our project.

The literature search will address the particles emitted by farms, sampling methods, (dispersion) modelling and virus viability. Regular meetings with peers will be scheduled and conferences will be attended. During the whole project, literature will remain to be evaluated to be able to take newly available data and techniques into account. To systematically evaluate and summarize the literature, and ensure the progress and stimulate the PhD student, a review paper will be written.

End product: literature review, framework for model selection and a work plan for the rest of the project

The duration of the work package will be six months.

Work package 2: Mathematical model selection/design

The main goal is to design a mathematical model framework that suits our main question. Therefore, the aim is to select mathematical models that can predict the spread of viable airborne particles between farms, considering:

1. The weather conditions that apply at a specific time
    a. Forward trajectory: so, predictions can be made at what farms will be at risk according to the weather forecast
    b. Back trajectory: to identify the farms that have been exposed in the past days

2. The spatial composition that may influence the airflow, like buildings, roads, trees, rivers
3. Virus viability in different matrices
4. Particle behaviour

The robustness of the framework must be sufficient to perform well under a variety of input parameters, for example, the differences in (the spatial composition of) the landscape between the coastal province of Friesland and the Gelderse Vallei, in the centre of the Netherlands, which are also a low and high farm density area, respectively.

For the modelling of the airborne spread between farms, the processes that play a role must be identified. In this case, these processes can be dived into three phases:

1. Generation and exhalation
2. Transport
3. Entry, deposition, and infection

Per phase, parameters that influence the viability of the virus need to be included in the model. In **figure 2**, these parameters are listed.

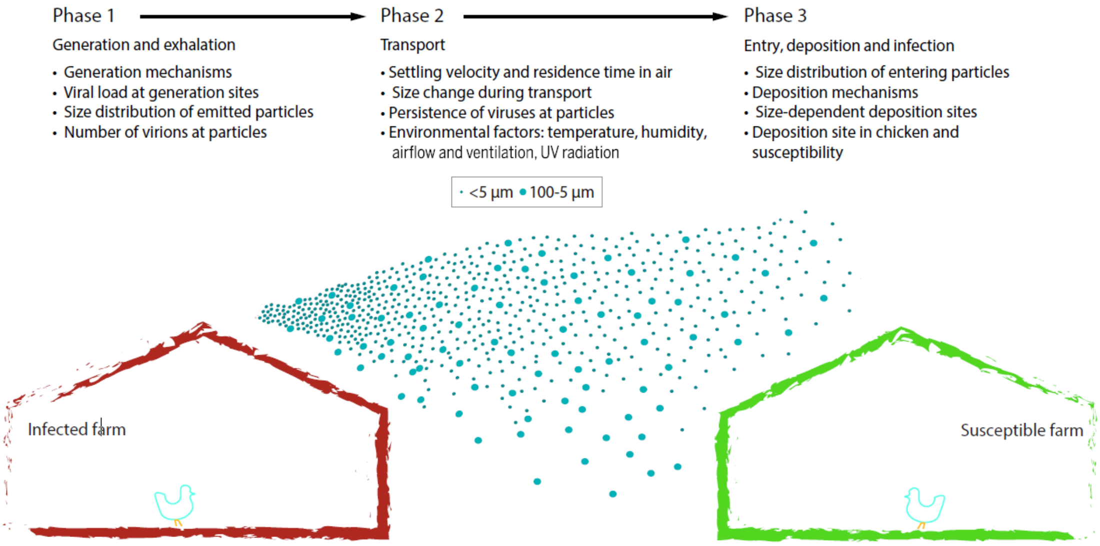


Figure 2: The phases and processes involved in the airborne transmission of AIV between farms. Red farm represents an infected farm, the green farm a susceptible farm. The dots illustrate the particles involved in airborne transmission. Figure is adapted from Wang et al. (2021).

The design: To design the model framework that can predict and simulate the behaviour of particles that may be transmitted between farms, the input parameters need to be defined and the desired quality of the output data defined. First, the input parameters will be specified. Second, the mathematical models that have been collected from the literature in work package 1, will be evaluated, run with historical data and if needed adapted. The findings will be integrated into the design of experiments that will be carried out in work package 3. In this work package the unknown parameters will be determined. The best model, or combination of models, will be used for further risk assessment.
End product is a model framework that can consist of one model or a combination of models
The duration of this work package will be six months.

Types of models used in AIV transmission
Models that have the potential to fit our data are ranked below. This is a pre-collection to help design work package 3. Additions to this selection will be made during the project. The models are ranked, to what we believe will suit best.

Atmospheric dispersion modelling (ADM) is an often-used model in air pollution research (Heederik et al., 2019) and could be used for this project. The model assumes three transport mechanisms of particles in the air: wind/advection, diffusion/advection and gravity/deposition. The diffusive and advective processes form a bell-shaped curve are plotted into a graph (y= concentration and x= space). This bell-shaped curve resembles a gaussian distribution. If transferred into a 3D model, the distribution forms a plume shape, hence called the plume model, see **figure 3**. This model type has been used in several studies (Jonges et al., 2015; Ssematimba et al., 2012) for the modelling of airborne transmission of AIV.

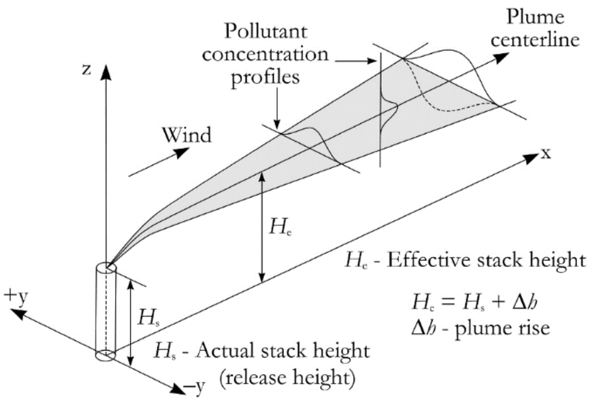


Figure 3: The Gaussian plume model, the key parameters are depicted in the figure (Leelőssy et al., 2014).

Computation fluid dynamics (CFD) modelling is a type of modelling that takes wind flow, wind velocities, turbulence, wind tunnels and the temperature into account. It is often used in determining airflows in and around buildings, for example to predict the effect of a new ventilation system. This type of modelling was used to determine the risk of residents near LPM's in an area with lots of complex buildings (Wei et al., 2018). The study showed that this type of modelling could predict the direction of particle deposition under these circumstances. This type of model could be used in addition or in combination with for instance AMD modelling.

HYSPLIT modelling
Model can be used for backward and forward trajectory modelling. It is often used as a forecast for air pollution. The air trajectory is an estimate of air mass movement over space and time. This type of model was used previously (Zhao et al., 2019) with the use of input parameters from literature. The concentration of airborne virus was estimated for PM10 and PM2.5. As the authors argue themselves, the input parameters could have caused extreme/worst-case scenarios when the parameters were at ceiling values. For input parameters, see the supplementary data of this manuscript (Zhao et al., 2019).

Example of a model used in COVID-19 pandemic
Contagion Airborne Transmission (CAT) modelling
The model is inspired by the Drake equation that was used to predict the number of beings in our galaxy that we potentially could communicate with. It is built out of probabilistic factors that, when multiplied, result in a total rate of (in the case of infectious particles) viable virions inhaled by the susceptible host. The CAT model is designed to estimate the risk of airborne transmission of COVID-19 but could be adapted to farms emitting infectious influenza virus. It is based on the 3-phase sequence of processes that is described in **figure 2**. In **figure 4** the equation with input parameters are depicted. Adaptations needs to be made in terms of particle types and sizes (Mittal et al., 2020) before the model can be applied.

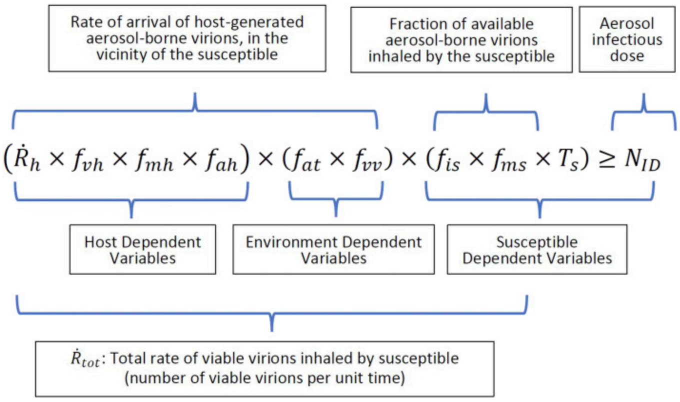


Figure 4: the equations of the CAT model and explanations of the input parameters (Mittal et al., 2020).

Work package 3: Experiments
The main goal is to generate the input variables that can be used in work package 4: mathematical models. Work package 3 will include pilot experiments, experiments in the lab and field experiments. The design stated here is based on expectations of what is needed beforehand but will be further specified based on the findings in work packages 1, 2 and 3.
The design: we will address the questions regarding the missing input parameters as described below.

First, experiments will be done in the lab, addressing the following questions/gaps

1. The behaviour of feathers, feather parts and organic materials
    a. This will be tested by performing simulations of these particles under airflows that are similar to exhaust ventilation rates

   b. Conditions will be varied: temperature, air humidity, wind direction, wind force, UV-radiation, pH
2. The viability of HPAIV strain H5N1 when attached to feather material and faeces
   a. In a special biosafety level 3 laboratory, AIV of a known dose will be applied to feather material and faeces. The survival will be tested under several conditions: temperature, air humidity, wind direction, wind force, UV-radiation, pH
3. Sampling devices for the field experiments will be tested. Important is that these samplers can collect particles ranging from fine particle matter to feather materials. Next to this, it is important that the viability of the virus is compromised as least as possible
   a. A set of samplers will be exposed to the same mixture of particles as described previously (Raynor et al., 2021)
   b. The mixture is 'infected' with a known dose of infectious virus. Infectious particles will be generated as described previously (Sedlmaier et al., 2009).
4. Recovery of viral RNA and viable virus from different types of sampled material.

Aerosol technology

For designing a sampling strategy in laboratory and field experiments, knowledge about aerosol technology is crucial. The first point of attention is the aerodynamic diameter of an aerosol, which is defined as that of a sphere with a density of 1 g cm −3 (cf. density of water) (Hinds & Zhu, 2022). This parameter together with the Stokes equivalent (considering a sphere as well) are often used in mathematical models. However, the particles emitted by poultry houses are of irregular shape, influencing behaviour in the air, like the settling velocity, see **figure 5.** Irregular shaped particles tend to settle more slowly than their equivalent volume spheres (Hinds & Zhu, 2022), influencing their residence time in the air. Residence time in the air depends on the aerosol diameter as depicted in **figure 6**. This estimation is based on Stokes' law for spherical particles, and as indicated above, will be different for the particles emitted by poultry farms. Next to this, as depicted in the figure, the exhaust height of poultry farms is of influence on residence time in the air as well and needs to be taken up in the model and integrated into the field experiments by determining the air sampler heights.

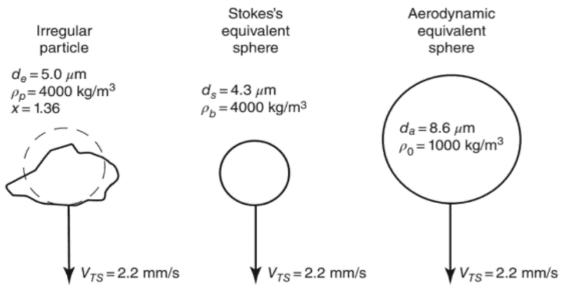


Figure 5: Three different particle with the same settling velocity but different shapes, diameters, and density. Figure is taken from Hinds & Zhu (2022).

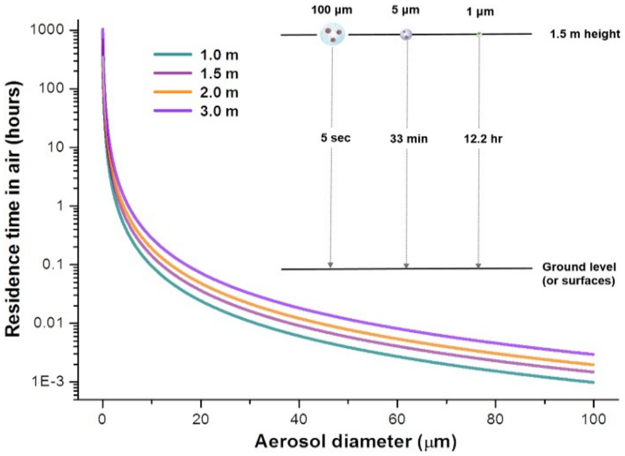


Figure 6: The time aerosols stay in the air for a range of sizes emitted at various heights above ground level, estimated by Stokes' law for spherical particles. Figure is taken from Wang et al. (2021).

Sample methods

Sampling small particles diluted into air is difficult. Several methods have been developed, but no standard methods with the desired level of performance have been developed yet. As discussed in the *background, subsection sampling methods*, the results are influenced by the sample device used. Methods that are available are depicted in **figure 7** and summarised in **table 1** (Pan et al., 2019).

The transport and capture of particles in samplers is dependent on particle size subjected to several mechanisms that influence the sensitivity of the sampler. Not all small particles will be captured, since these diffuse due to Brownian motion; medium sized particles travel with the airflow and are efficient to capture based on their particle inertia, whereas large particles tent to settle due to gravity. The design of air samplers and air sampler inlet and calculation of particle losses for the chosen system are important (Pan et al., 2019; von der Weiden et al., 2009). That the sample method is of influence for the results was shown previously (Raynor et al., 2021). Raynor et al. (2021) compared several samplers that were exposed to the same mixture at the same time under the same circumstances. The particle sizes recovered showed variation, whereas this should have been similar if the samplers would have performed equally. The distribution is shown in **figure 8.**

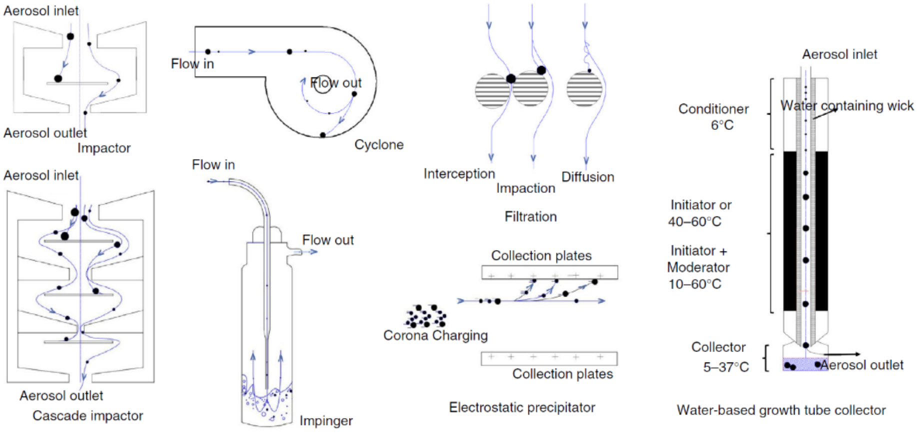
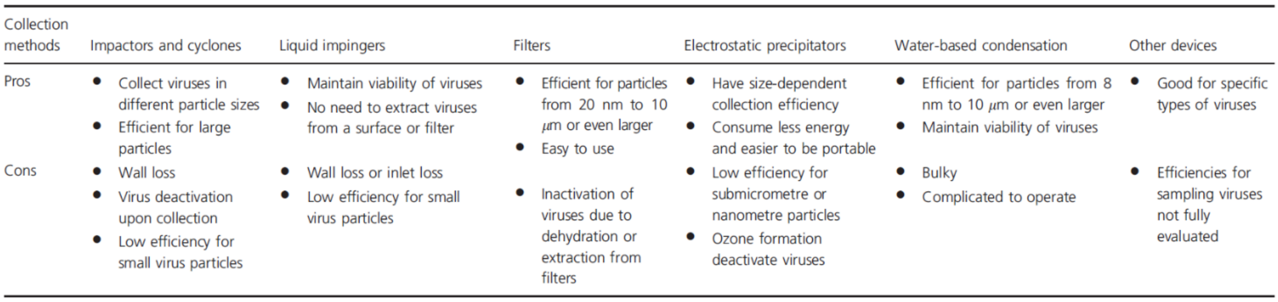


Figure 7: Airborne virus samplers. Schematic picture of the common samplers available and their mechanisms. Figure is taken from Pan et al. (2019).



Collection methods	Impactors and cyclones	Liquid impingers	Filters	Electrostatic precipitators	Water-based condensation	Other devices
Pros	• Collect viruses in different particle sizes • Efficient for large particles	• Maintain viability of viruses • No need to extract viruses from a surface or filter	• Efficient for particles from 20 nm to 10 μm or even larger • Easy to use	• Have size-dependent collection efficiency • Consume less energy and easier to be portable	• Efficient for particles from 8 nm to 10 μm or even larger • Maintain viability of viruses	• Good for specific types of viruses
Cons	• Wall loss • Virus deactivation upon collection • Low efficiency for small virus particles	• Wall loss or inlet loss • Low efficiency for small virus particles	• Inactivation of viruses due to dehydration or extraction from filters	• Low efficiency for submicrometre or nanometre particles • Ozone formation deactivate viruses	• Bulky • Complicated to operate	• Efficiencies for sampling viruses not fully evaluated

Table 1: Overview of the pros and cons of the samplers depicted in **figure 7**. Table is taken from Pan et al. (2019).

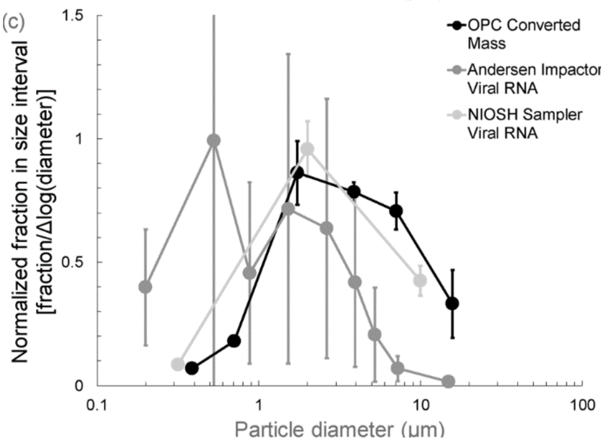


Figure 8: Size distribution of AIV particles in log scale for the Anderson Impactor, NIOSH sampler and Optical Particle counter (OPC). Figure is taken form Raynor et al. (2021).

The following points must be considered in designing the field experiments

1. The use of high flow vs low flow samplers.
   High flow rate samplers have a lower detection limit compared to low flow rate samplers and are therefore the sampler of choice for measuring low virus concentrations. However, these samplers influence the virus viability, underestimating the infectious potential (Pan et al., 2019; Raynor et al., 2021)
2. Particle size discrimination
   Samplers vary in sensitivity to particle fractions recovered (Raynor et al., 2021)
3. Recovery of viable virus particles
   Sampling methods can damage the particles resulting in non-viable virus.
4. Sampling time
5. Stationary vs mobile samplers

These factors will all be considered when testing promising sampling strategies in the lab and taken up in the field experiment design. The sampler testing setup will be similar to the design described by Raynor et al., (2021) using a dual approach as suggested (Raynor et al., 2021). This dual approach was recommended: the high flow sampler for sampling virus with low concentrations and the low flow sampler to obtain infectious virus.

Second, field experiments will be performed, and input variables for work package 4 will be collected.
The criteria for poultry farms to be selected are expected to be:

- Indoor laying hen farm → because this is the most common type of farm in areas with high density of farms and the majority of HPAIV infected poultry farms are laying hen farms with chickens housed inside (because of the housing order).
- Farms in various stages of infection will be accepted to the study. This because all the stages are relevant for the Dutch situation
  - If possible, sampling will be done at farms before culling. This however will be difficult because culling follows almost immediately after positive diagnosis.
  - During culling
  - After culling
  - Farms in the 1km zone around the farm
  - Farms that turn out to be negative after sampling will serve as control farms
- Farms need to be infected with HPAIV, since viability is different between LPAIV and HPAIV

Data that needs to be gathered

- Weather circumstances
  - Temperature, relative humidity (RH), UV-index, wind direction, wind force, sun hours, precipitation. These are all of influence on the virus survival and particle deposition.
    - Data will partly be requested from the KNMI (wind direction, wind force, UV-index) and partly measured during the experiments (RH, temperature)
- Information about the spatial composition will be requested by the land registry for the nearby building, roads etc. Information of the farm will be recorded during the experiments.
- Sample locations
  - In the poultry house: to know the starting concentration
    - Number of particles per particle fraction/size with, the virus RNA and viable virus
  - External exhaust fans: to know wat concentration is emitted into the environment
    - Number of particles per particle fraction/size with, the virus RNA and viable virus
  - Downwind
    - Number of particles per particle fraction/size with, the virus RNA and viable virus
    - We will try to collect viable virus to determine whether and over what distances viable virus can be spread via the air. However, we expect that the success of collection of viable virus will not be representative for the actual situation because the concentrations are likely under the limit of detection. In that case, for the modelling, we will use estimates of the virus concentration as input variable based on data regarding viral decay. To correct for the uncertainty, various scenarios will be simulated.

The end product is input data for model simulations and data on particle types and infectious virus.
Duration of this work package will be two years.

Work package 4: Model simulations

The main goal is to develop a basic model that can be used as a risk assessment tool by the government and policy makers. The chosen model(s) will be simulated with the obtained data under various circumstances. This to test how dependent the outcome is of the input parameters. The model will be fine-tuned based on the simulation outcomes, and by including new data when available. The goal is to develop a model that is robust to variation in input variables and shows a good fit with available data.
The end product is a basic output model that can be used for risk assessment purposes by the government, policymakers and researchers.
Duration: will be one year.

B.2.3 Feasibility / Risk assessment

There are several risks that affect the feasibility and relevance of the end products of the project as currently proposed. These risks can be divided into experimental risks and modelling risks.
One of the experimental risks is that there will be no (HP)AIV outbreaks during the project or that there will be no more poultry in the Netherlands. In the unlikely event that this would occur, we can still obtain enough missing parameters with simulation experiments in the lab to improve the risk assessment. Another option could be for us to go abroad to perform field experiments. The results are still of scientific importance because (HP)AIV is a worldwide problem. Another risk could be that a vaccination campaign is implemented for poultry in the Netherlands, which could change the epidemiology. However, vaccination will not be able to completely prevent the virus from spreading, and most likely, the transmission route through the air would remain important, for both neighbouring poultry farms and residents/occupants. Therefore, adaptations to the model to fit the changed epidemiology will still make the developed model applicable.

In addition to the experimental risks, the mathematical model may not be accurate/robust enough to estimate the risk under varying conditions or under all relevant conditions. It Is likely however, that the model will be applicable for specific areas or for specific (limited) situations. In that case, the model is still of value for those conditions. Besides this, this would contribute to our understanding of what does not work in modelling dispersion of infectious virus. In addition, the experimental results can still be used by other researchers as input parameters.

B.2.4 Scientific (a) and societal (b) impact

The results of this project will be of impact for both the scientific community and the society. The results will help us with the understanding of the airborne spread of AIV and will allow for a better risk assessment. It will contribute to sustainable control measures that can be targeted at farms that are at high risk to airborne transmission. These measures can be more accurately focused on the 'high risk' particles to limit the airborne spread and epidemics will be shortened in duration and the total number of animals culled will be limited. This will make a huge difference to the high costs that are now being made by the sector for dealing with ongoing outbreaks. With less outbreaks, free range chickens can experience the outside (more) and fewer birds will suffer and die horrible deaths. Another important point is ensuring the food supply worldwide, which could come under pressure if these outbreaks continue or worsen.

For public health, AIV is of zoonotic treat and has the potential to cause pandemics. Limiting outbreaks will limit the exposure to humans. The risk for human transmission for the variant that is currently circulating (October 2022) is not high (Adlhoch et al., 2022). However, virus adaptation to the mammalian body could happen, or zoonotic variants circulating elsewhere could be introduced. The risk model that will be developed in this project allows us to better estimate the risk of exposure to humans (residents, occupants) in these cases. If the risk is estimated as high, proper control measures can be designed to prevent spill over. The model will help us evaluate the current poultry farm 'landscape' and explore possibilities in relocating farms to areas with low densities of poultry farms and residents. Beside this, another PhD student is working on modelling avian-human transmission. The data generated with this project is very useful for the avian-human transmission project.

Some farmers are already experimenting with ways to prevent 'infectious' particles getting in and out the farm. These experiments are considering ventilation strategies, sometimes at high cost without any indication of efficacy, an example of this is the installation of windbreak mesh. Pilot studies, carried out by the Royal GD, are looking into the effectiveness and results are promising (van Spanjen, 2022). The understanding of particle types and infectious virus will help us to determine the requirements for windbreak mesh, how it should be placed, the mesh size etc. Particles can then be tackled before they exit and enter farms, reducing transmission between farms and virus introduction from wild bird sources. When these kinds of measures are proven to be effective, it is less of a gamble for farmers whether it can be effective or not, which will ensure that this will be implemented by more farmers.

The behaviour of particles in the air is relevant for many fields of research. It will be useful for all farm related infectious diseases, since farms emit irregular shaped particles. Beside this, it will be useful for modelling of infectious diseases, particulate emissions by industry, roads and wood stoves etc, and it is relevant to physics and fluid dynamics.

In short, AIV is a major problem that costs a lot of suffering and money. In addition, the risk of a pandemic is lurking. A better understanding of the airborne spread will help reduce the severity of the outbreaks and protect public health.

B.2.5 Ethical considerations

Permission will be requested from landowners and farmers for the air measurements.

B.2.6 Literature/references

Aarnink, A., Cambra-López, M., Lai, H., & Ogink, N. (2011). *Deeltjesgrootteverdeling en bronnen van stof in stallen [samenvattende reportage]* (No.452). Wageningen UR Livestock Research. http://edepot.wur.nl/171536

Adlhoch, C., Fusaro, A., Gonzales, J. L., Kuiken, T., Marangon, S., Niqueux, É., Staubach, C., Terregino, C., Guajardo, I. M., Chuzhakina, K., & Baldinelli, F. (2022). Avian influenza overview June – September 2022. *EFSA Journal*, *20*(10). https://doi.org/10.2903/j.efsa.2022.7597

Alexander, D. J. (2000). A review of avian influenza in different bird species. *Veterinary Microbiology*, *74*(1), 3–13. https://doi.org/https://doi.org/10.1016/S0378-1135(00)00160-7

Alonso, C., Raynor, P. C., Davies, P. R., & Torremorell, M. (2015). Concentration, size distribution, and infectivity of airborne particles carrying swine viruses. *PLoS ONE*, *10*(8). https://doi.org/10.1371/journal.pone.0135675

Alonso, C., Raynor, P. C., Goyal, S., Olson, B. A., Alba, A., Davies, P. R., & Torremorell, M. (2017). Assessment of air sampling methods and size distribution of virus-laden aerosols in outbreaks in swine and poultry farms. *Journal of Veterinary Diagnostic Investigation*, *29*(3), 298–304. https://doi.org/10.1177/1040638717700221

Bodewes, R., & Kuiken, T. (2018). *Chapter Twelve - Changing Role of Wild Birds in the Epidemiology of Avian Influenza A Viruses* (M. Kielian, T. C. Mettenleiter, & M. J. Roossinck, Eds.; Vol. 100, pp. 279–307). Academic Press. https://doi.org/https://doi.org/10.1016/bs.aivir.2017.10.007

Bordes, L., Vreman, S., Heutink, R., Roose, M., Venema, S., Pritz-Verschuren, S. B. E., Rijks, J. M., Gonzales, J. L., Germeraad, E. A., Engelsma, M., & Beerens, N. (2022). Highly pathogenic avian influenza H5N1 virus infections in wild red foxes (Vulpes vulpes) show neurotropism and adaptive virus mutations. *BioRxiv*, 2022.07.21.501071. https://doi.org/10.1101/2022.07.21.501071

Filaire, F., Lebre, L., Foret-Lucas, C., Vergne, T., Daniel, P., Lelièvre, A., de Barros, A., Jbenyeni, A., Bolon, P., Paul, M., Croville, G., & Guérin, J.-L. (2022). Highly Pathogenic Avian Influenza A(H5N8) Clade 2.3.4.4b Virus in Dust Samples from Poultry Farms, France, 2021. *Emerging Infectious Diseases*, *28*(7), 1446–1450. https://doi.org/10.3201/eid2807.212247

Gambotto, A., Barratt-Boyes, S. M., de Jong, M. D., Neumann, G., & Kawaoka, Y. (2008). Human infection with highly pathogenic H5N1 influenza virus. *The Lancet*, *371*(9622), 1464–1475. https://doi.org/10.1016/S0140-6736(08)60627-3

Germeraad, E. A., Beerens, N., Elbers, A. R. W., & Slaterus, R. (2022). *"Rapid Risk Assessment" voor introductie van hoog pathogene aviaire influenza in de Nederlandse commerciële pluimveehouderij*. https://library.wur.nl/WebQuery/wurpubs/589772

Germeraad, E. A., Sanders, P., Hagenaars, T. J., de Jong, M. C. M., Beerens, N., & Gonzales, J. L. (2019). Virus shedding of avian influenza in poultry: A systematic review and meta-analysis. *Viruses*, *11*(9). https://doi.org/10.3390/v11090812

Heederik, D., Erbrink, H., Farokhi, A., Hagenaars, T., Hoek, G., Ogink, N., de Rooij, M., Smit, L., Winkel, A., & Wouters, I. (2019). *Risicomodellering veehouderij en gezondheid (RVG): modellering van regionale endotoxineconcentraties en relaties met gezondheidseffecten*. (IRAS UU; No. 2019-01), (WBVR; No. 1910304). Wageningen University & Research. https://doi.org/10.18174/470543

Hinds, W. C., & Zhu, Y. (2022). *Aerosol technology: properties, behavior, and measurement of airborne particles*. John Wiley & Sons.

Jonges, M., van Leuken, J., Wouters, I., Koch, G., Meijer, A., & Koopmans, M. (2015). Wind-mediated spread of low-pathogenic avian influenza virus into the environment during outabreaks at commercial poultry farms. *PLoS ONE*, *10*(5). https://doi.org/10.1371/journal.pone.0125401

Karunakaran, A. C., Murugkar, H. v, Kumar, M., Nagarajan, S., Tosh, C., Pathak, A., Mekhemadhom Rajendrakumar, A., & Agarwal, R. K. (2019). Survivability of highly pathogenic avian influenza virus (H5N1) in naturally preened duck feathers at different temperatures. *Transboundary and Emerging Diseases*, *66*(3), 1306–1313. https://doi.org/https://doi.org/10.1111/tbed.13148

Leelőssy, Á., Molnár, F., Izsák, F., Havasi, Á., Lagzi, I., & Mészáros, R. (2014). Dispersion modeling of air pollutants in the atmosphere: a review. *Central European Journal of Geosciences*, *6*(3), 257–278. https://doi.org/10.2478/s13533-012-0188-6

Ministerie van Algemene Zaken. (2022a, October). *Besmettingen en ruimingen vogelgriep*. Retrieved October 16, 2022 from https://www.rijksoverheid.nl/onderwerpen/vogelgriep/besmettingen-vogelgriep-bij-bedrijven

Ministerie van Algemene Zaken. (2022b, October 6). *Landelijke maatregelen bij uitbraak vogelgriep | Vogelgriep | Rijksoverheid.nl*. Retrieved October 16, 2022 from https://www.rijksoverheid.nl/onderwerpen/vogelgriep/maatregelen-tegen-vogelgriep

Mittal, R., Meneveau, C., & Wu, W. (武文). (2020). A mathematical framework for estimating risk of airborne transmission of COVID-19 with application to face mask use and social distancing. *Physics of Fluids*, *32*(10), 101903. https://doi.org/10.1063/5.0025476

Pan, M., Lednicky, J. A., & Wu, C.-Y. (2019). Collection, particle sizing and detection of airborne viruses. *Journal of Applied Microbiology*, *127*(6), 1596–1611. https://doi.org/https://doi.org/10.1111/jam.14278

Raynor, P. C., Adesina, A., Aboubakr, H. A., Yang, M., Torremorell, M., & Goyal, S. M. (2021). Comparison of samplers collecting airborne influenza viruses: 1. Primarily impingers and cyclones. *PLoS ONE*, *16*(1 January). https://doi.org/10.1371/journal.pone.0244977

Scoizec, A., Niqueux, E., Thomas, R., Daniel, P., Schmitz, A., & le Bouquin, S. (2018). Airborne detection of H5N8 highly pathogenic avian influenza virus genome in poultry farms, France. *Frontiers in Veterinary Science*, *5*(FEB). https://doi.org/10.3389/fvets.2018.00015

Sedlmaier, N., Hoppenheidt, K., Krist, H., Lehmann, S., Lang, H., & Büttner, M. (2009). Generation of avian influenza virus (AIV) contaminated fecal fine particulate matter (PM2.5): Genome and infectivity detection and calculation of immission. *Veterinary Microbiology*, *139*(1–2), 156–164. https://doi.org/10.1016/j.vetmic.2009.05.005

Shinya, K., Ebina, M., Yamada, S., Ono, M., Kasai, N., & Kawaoka, Y. (2006). Influenza virus receptors in the human airway. *Nature*, *440*(7083), 435–436. https://doi.org/10.1038/440435a

Spackman, E. (2008). A Brief Introduction to the Avian Influenza Virus. In E. Spackman (Ed.), *Avian Influenza Virus* (pp. 1–6). Humana Press. https://doi.org/10.1007/978-1-59745-279-3_1

Ssematimba, A., Hagenaars, T. J., & de Jong, M. C. M. (2012). Modelling the wind-borne spread of highly pathogenic avian influenza virus between farms. *PLoS ONE*, *7*(2). https://doi.org/10.1371/journal.pone.0031114

Swayne, D. E., & Suarez, D. L. (2000). Highly pathogenic avian influenza. *Revue Scientifique et Technique-Office International Des Epizooties*, *19*(2), 463–482. https://doi.org/10.20506/rst.19.2.1230

van Spanjen, M. (2022, October). *Windbreekgaas veelbelovende maatregel om het risico op vogelgriepbesmettingen via de lucht te verkleinen*. Retrieved October 16, 2022, from https://www.avined.nl/nieuws/windbreekgaas-veelbelovende-maatregel-om-het-risico-op-vogelgriepbesmettingen-via-de-lucht-te-verkleinen

von der Weiden, S.-L., Drewnick, F., & Borrmann, S. (2009). Particle Loss Calculator-a new software tool for the assessment of the performance of aerosol inlet systems. *Atmospheric Measurement Techniques*, *2*(2), 479–494. https://doi.org/10.5194/amt-2-479-2009

Wang, C. C., Prather, K. A., Sznitman, J., Jimenez, J. L., Lakdawala, S. S., Tufekci, Z., & Marr, L. C. (2021). Airborne transmission of respiratory viruses. *Science*, *373*(6558). https://doi.org/10.1126/science.abd9149

Wei, J., Zhou, J., Cheng, K., Wu, J., Zhong, Z., Song, Y., Ke, C., Yen, H. L., & Li, Y. (2018). Assessing the risk of downwind spread of avian influenza virus via airborne particles from an urban wholesale poultry market. *Building and Environment*, *127*, 120–126. https://doi.org/10.1016/j.buildenv.2017.10.037

Winkel, A., Mosquera, J., Groot Koerkamp, P. W. G., Ogink, N. W. M., & Aarnink, A. J. A. (2015). Emissions of particulate matter from animal houses in the Netherlands. *Atmospheric Environment*, *111*, 202–212. https://doi.org/10.1016/j.atmosenv.2015.03.047

Ypma, R. J. F., Jonges, M., Bataille, A., Stegeman, A., Koch, G., van Boven, M., Koopmans, M., van Ballegooijen, W. M., & Wallinga, J. (2013). Genetic data provide evidence for wind-mediated transmission of highly pathogenic avian influenza. *Journal of Infectious Diseases*, *207*(5), 730–735. https://doi.org/10.1093/infdis/jis757

Zhao, Y., Richardson, B., Takle, E., Chai, L., Schmitt, D., & Xin, H. (2019). Airborne transmission may have played a role in the spread of 2015 highly pathogenic avian influenza outbreaks in the United States. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-47788-z