Title:

# Assessing the risk of highly pathogenic avian influenza H5 viruses spread within French broiler and layer production networks.

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# Abstract

Since 2015, French poultry production is threatened almost every year by a reintroduction of highly pathogenic avian influenza H5 viruses. The duck sector was the most concerned by this crisis but other sectors such as broiler, layer and turkey were also affected by outbreaks. The objective of this work was to assess the risk of highly pathogenic avian influenza H5 virus transmission from one farm to another within the French broiler and layer production network.

This study used the WOAH risk assessment framework. After drawing up a scenario tree of virus transmission from one farm to another, data were collected through a literature review or through experts' elicitation. Three questionnaires were developed according to the experts' field of expertise: avian influenza, broiler and layer sectors. The experts' estimates were combined using a beta distribution weighted by their confidence level. A Monte Carlo iteration process was used to combine the different probabilities of the scenario tree and to assess the transmission risk.

In the broiler sector, the highest transmission probabilities were observed if the exposed farm was an indoor broiler farm and the source a broiler farm (indoor or free-range). The high transmission probability between broiler farm integrated within the same company highlighted the role of integration in this probability. In the layer sector, the highest transmission probabilities were observed if the source farm was a free-range farm and the exposed farm a production farm (indoor or free-range). The sensitivity analysis showed that the exposed farm's biosecurity had a significant impact on the transmission probability.

Our results provide an insight on the role of each type of farms in the virus spread within the French broiler and layer production network and will be useful for the implementation of control measures such as movement restriction or vaccination.

# Introduction

Since 2015, France has faced several epizootic waves of highly pathogenic avian influenza (HPAI) viruses [1–3]. The main poultry production sector affected by these waves has been the fattening duck production sector – for the production of duck liver-based delicacies called “foie-gras” [4,5]. During these waves, while the initial introduction of HPAI viruses has likely been due to wild birds [1,6], the large spread of the viruses between poultry farms was linked with the poultry production systems. The structure of the fattening duck sector was one of the main factors facilitating the spread of HPAI [6]. Indeed, in this sector, live birds’ movements occur between the different production stages: rearing, breeding and force-feeding. It has been demonstrated that these movements have played a major role in the spread of HPAI viruses [7,8].

Other poultry production sectors exist in France: the meat sector (broiler, meat duck, turkey, guinea fowl) and layer sector [9]. In the broiler production sector, birds enter the farm as day-old chicks and leave the farm to go to the slaughterhouse. In the layer sector, production is organized in two stages: pullet (from day-old chick to ready-to-lay layers) and layer (from ready-to-lay layers to spent hen). In between these two stages, live bird movements take place to transport ready-to-lay layers from the pullet farm to the layer farm. The same movement pattern is observed for breeders of all species. A live bird movement occurs between the growth stage farm and the fertile adult stage farm. Even if there are no live bird movements between broiler or layer farms, other

avian influenza (AI) virus pathways such, as fomite or human movements, may exist [10]. Identified fomite movements may occur through birds pick-up to slaughter, feed delivery, eggs collection, manure and litter management, dead bird management and shared equipment [11–15]. Movements involving direct contacts between humans and birds are identified as presenting the higher risk of AI virus spread (veterinarian, staff working on multiple premises, company staff, technical advisors etc.) [16,17].

Few risk assessment studies looked at the impact of the integration level (integrated or independent) or farming conditions (indoor vs. free-range) on the risk of AI viruses introduction in a farm [13,15,18]. Moreover, a few modelling studies collected data on the frequency of fomite and human movements between farms [11,14]. Nevertheless, these studies never took a close look at the origin of these movements, i.e. type of farm from which the movement originated.

So far, no study has been conducted on the risk of AI introduction into a chicken farm from another farm within the chicken production sector in France, even though these sectors have also been strongly affected by outbreaks (329 outbreaks in chicken farms during the 2020-22 period) [2]. The aim of this study was to assess the risk of spread of AI virus within two other French poultry production sectors: broiler and layer. This risk assessment study has been conducted considering different types of farms as described in a previous study [9]. These types of farms were defined according to the sector (broiler and layer)), production type (i.e. future breeders, breeders, production), the farming conditions (i.e. indoor and free-range) and the integration level of the farm. For this purpose, this study assesses the risk of transmission from one farm of a defined type to another farm of the same or different type within the same production sector. It did not consider the transmission risk between farms from different poultry sectors. The current study focuses on the relative transmission risk, i.e. to identify the

type of farms with the highest risk of virus transmission through indirect contacts (human and fomites movements). This study will allow us to identify pathways and farm types most at risk of AI virus transmission.

# Materials and methods

## Risk assessment model

This study used the risk analysis methodology described by the WOAH [19] to conduct a partial risk assessment of the likelihood of an AI virus entering a flock. As the aim of the study was to measure the relative risk of viral spread between different types of farms, we focused on the risk of introduction. We assumed that once the virus is introduced into the flock, the probability of a bird being infected, as well as the risk of exposure, is close to 100%, and we did not assess the consequences of AI spread. The virus introduction through the live bird movement pathway from an infected farm to a free farm was considered very high. Since we have assumed that the introduction of at least one infected bird into a free farm makes the farm infected, our risk assessment study has focused solely on the risk of introduction through indirect pathways within a poultry production network. These pathways were identified in a previous literature scoping review [10]: human with a direct contact with birds; trucks transporting live birds; trucks transporting feeds, manure and dead birds; shared materials in direct contact with birds (Figure 1). Two other pathways known to represent a very low risk and thus were not considered: human and shared materials with no direct contact with birds or manure [11,15,20]. Environmental pathways such as air-born or rodent as mechanical vector transmission pathways were not assessed as the study was conducted under the hypothesis that these environmental pathways

would be similar between all types of farms. Geographical proximity between farms was not considered either.

Figure 1: The transmission pathways considered in this risk-assessment study.

The assessment was conducted for the broiler and layer sectors on several types of farms defined in Table 1 and adapted from the description provided by Hautefeuille et al. [9]. Considering the low number of breeder farms and future layers farms, we did not include the integration level for these types of farms.

Table 1: Listing of the different types of farms studied according to the production type, the farming conditions and the integration level of farms in the French broiler and layer production sector

| Production type | Farming conditions | Integration level |
|---|---|---|
| Broiler | Indoor | integrated with a hatchery |
| | | integrated without any hatchery |
| | | independent |
| | Free-range | integrated with a hatchery |
| | | integrated without any hatchery |
| | | independent |
| Breeder broiler | Indoor | - |
| Future breeder broiler | | - |
| Future layer | Indoor | - |

| | | |
|---|---|---|
| Layer | Indoor | integrated |
| | | independent |
| | Free-range | integrated |
| | | independent |
| Breeder layer | Indoor | - |
| Future breeder layer | | - |

## Data source

The probability of virus introduction from each pathway was calculated using scenario trees (Supplementary file 1), as recommended by EFSA [21]. The first step of this study was to identify, in the scenario trees, the branches for which estimates of risk probabilities could be obtained from the French legislation (Supplementary file 1). The second step was to generate data for each pathway on the transmission risk, the frequency of occurrence for one type of farm and the probability that this pathway exists between two different types of farms. An expert elicitation survey was conducted to obtain the data not available from the scientific literature.

## Experts' elicitation survey

The aim of this survey was to collect data on the transmission risk for each type of farms (8 farm types for broiler and 7 for layer production) and for each pathway (9 different pathways). We decided to create a survey with three questionnaires, each of which was a reasonable length for respondents while still collecting all of the necessary data. Each questionnaire was intended for one type of expert profile: one for experts on AI epidemiology and poultry farm biosecurity, one for experts on French broiler

sector and one for experts on French layer sector. In our study, experts were defined as persons with relevant experience on one of the different topics studied.

Experts were recruited according to their experience on the different questionnaire topics. Experts requested at first for the questionnaire on AI were scientists from diverse national French research centres currently working and publishing on AI in France. Experts requested at first for the questionnaire on broiler or layer sector are national representatives of the French broiler or layer sector. Following this initial recruitment, a “snowball” recruitment was conducted, in which experts who participated in the survey were asked to recommend other experts to fill out the questionnaire. Experts filled the survey independently without being in contact with each other. The questionnaire was completed between June 1 and July 31, 2020.

The questionnaire was designed online using the tool Survey Monkey (www.surveymonkey.com). For each questionnaire a pilot survey was sent to two experts working on the questionnaire’s field in order to test the survey and optimize it. In the aim to reduce the length of each questionnaire, some simplifications were decided and validated with these two experts (Table 2).

Table 2: Simplification made according to the probability and the pathway concerned and justification validated by two experts of the poultry production sector.

| Probability concerned | Pathway concerned | Simplification made | Justification |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Probability of virus introduction and release | Pullet transport<br>Birds to slaughter transport | Merging of the two pathways as pick-up transport | Pick-up of birds is conducted in a similar way for pullet and birds to slaughter. |
| Probability that the studied pathway occurred during the previous week in farm A (i.e. Probability of pathway origin) | Manure<br>Dead birds | Merge of the two pathways | Management similar for both pathways |
| | | No consideration of the integration level of farms | Management similar regardless the integration level |
| | Shared materials | | |

The experts were emailed a description of the study objectives and of what was expected for their participation with the link to the online survey. The beginning of the online survey included instructions and contact information of the administering researchers. As this study was based solely on expert elicitation and did not involve animal experimentation, it was not necessary to consult an animal research ethics committee. Completion of the questionnaire constituted authorization to use the data provided and informed consent. The survey was not anonymous in order to contact the experts for clarification, but all their answers were analysed and presented anonymously. Personal information was retained to identify the experts, but was not used for the analysis presented in this work. The questionnaires are available in French upon request.

Each questionnaire was designed to take about one hour to be filled by experts. Questionnaires started with a description of the experts and questions on their expertise level. The first questionnaire on AI epidemiology was divided in four sections: risk of AI virus introduction in a farm, risk of AI virus release from an infected farm, risk associated with wild birds, and risk of an infected farm going undetected. To simplify the questionnaire, risk was assessed per farm biosecurity level rather than farm type. To do so a biosecurity grid was built based on previous works on the biosecurity evaluation process [7,22] and guidelines developed by the French poultry technical institute (ITAVI) [23]. This grid was validated by two experts outside of the list of experts identified for the risk assessment and currently working on French poultry farms biosecurity. Five biosecurity levels were defined from very low to very high, and are described in the biosecurity grid (Supplementary file 2). For the five defined biosecurity levels, experts were asked about the probability of introduction or release of the virus for each risk pathway (poultry production related or wild birds related). At the end, the experts were asked to define the probability that an infected farm would go undetected. The second questionnaire aimed to collect data on broiler sector and had four sections: biosecurity level of poultry farms, frequency of between farm movements and material, and detection level of AI virus infection. To assess the biosecurity level of a studied farm type, experts were asked to distribute 100 farms of this type between the five different levels in order to have the percentage of farms per biosecurity level (Supplementary file 2). The second section aimed to identify for each introduction pathway and for each type of farm, the frequency that this pathway occurred and the probability that this pathway links the studied farm with another type of farms. The questionnaire concluded on the capacity of a farmer to detect an AI infection based on the type of farm. For question concerning probabilities, experts were asked for the most

probable, the minimum and the maximum values. For each question, experts were asked to provide their confidence level with a score from 1 to 5 (Table 3) and they could add comments. Finally, for the three questionnaires, the experts were asked some open-ended questions regarding factors that might have influenced their answers.

Table 3: Matching between confidence level and score

| Level of confidence | Score |
|---|---|
| Certain | 5 |
| Very confident | 4 |
| Moderately confident | 3 |
| Not confident | 2 |
| Uncertain | 1 |

## Data analysis

Data collected from the SurveyMonkey platform were saved in a database template developed in Microsoft Excel® version 2007. The data from these two questionnaires were used to elicit Beta distributions characterising the ensemble of experts' assessments on each probability of interest. For each probability of interest, the parameters of the Beta distribution were determined in terms of expected value and variance of the distribution, which were in turn calculated from the expert's responses as follows. For the question asking for the most probable, minimum and maximum values of the target probabilities, the expected value was set at the average of the most probable values weighted by the expert confidence level and the variance was

calculated using a formula (1) balancing a maximal variance $V_m$ and an average variance $V_i$ between experts.

$$var = V_i + \frac{(V_m - V_i)^3}{{V_m}^2} \qquad (1)$$

The maximal variance $V_m$ corresponds to the variance of a Uniform distribution with support over the maximal interval (i.e. the smallest interval containing all the intervals reported by the experts). The average variance $V_i$, by contrast, is the average of the variances of the Uniform distributions over each of the reported intervals. This variance calculation allows the representations of divergent opinions without reducing compatible assessments. For questions requiring only one interval of values (i.e. 21% to 40%, 41% to 60%, etc.), such as questions on between farm contact probabilities, the centre of the interval was considered as the most probable, and the boundaries as the minimum and the maximum value for each expert. The analysis was conducted with R (version 4.0.2).

The frequency of occurrence and the origin of some pathways did not need to be assessed by experts as these values depend on the production cycle. The egg transport was considered to enter into a given breeder farm every day and the possible origin of this truck was always breeder farms. The frequency of chick and pullet delivery and slaughter take off was defined by the production cycle of the different types of production. As it appears that experts could not provide an occurrence frequency for shared material, it was arbitrarily considered to occur once a month.

A Monte Carlo iterative process was carried out on the scenario trees described above, in order to combine the different probabilities and to calculate the transmission probability from one type of farm to another. The probability of realisation of the event

for all branches of the scenario tree were randomly sampled from the Beta distribution previously elicited. The simulation process used 10 000 iterations.

## Sensitivity analysis

A sensitivity analysis was conducted using the one-at-time method applied on the biosecurity level of the source and the exposed farms and on the pathways [24]. This analysis was applied only for two types of farms with the most extreme daily transmission probabilities: one representing the highest and one the lowest. This sensitivity analysis is based on the distribution of the transmission probabilities on 10 000 iterations.

# Results

## Experts' description

At the end of the process, six experts responded to the questionnaire on AI epidemiology. Among these experts, three had more than ten years of experience on AI, one more than five years and the last two more than three years. Seven experts responded to the questionnaire on the broiler sector and all of them had more than 10 years of experience on the broiler sector. Five experts responded to the questionnaire on the layer sector and all of them had over 10 years of experience on the layer sector.

## Probability of avian influenza virus transmission

Based on the survey results, we found that, for the broiler production network, the highest transmission probabilities are observed when the source farm is a production farm (i.e. not selection) (indoor or free-range) and the exposed farm is an indoor broiler farm, regardless of the integration level (Table 4). Transmission probabilities are lowest when the exposed farm is a selection farm or when the source farm is a selection farm and the exposed farm a free-range farm (without consideration of the integration level).

For the layer production network, the highest transmission probabilities are observed when the source farm and the exposed farm are production farms (pullet, indoor or free-range layer) (Table 5). Transmission probabilities are lowest when the source farm and/or the exposed farm are selection farms.

Table 4: Daily transmission probabilities of HPAI H5 virus between an infected source farm and an exposed farm (free of infection) according to the type of farms in the French broiler production network (median, Q25 and Q75 values of 10 000 iterations). Percentage of transmission probabilities below the transmission probability between independent indoor broiler farms: Dark brown: 50-60%, brown: 60-75%, medium brown: 75-85%, light brown: 85-95%, no colour: over 95%.

| **Values in %** | | **Exposed farm (free of infection)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Indoor broiler | | | Free-range broiler | | | Selection | |
| **Source farm (infected)** | | Integrated with hatchery | Integrated without hatchery | Independent | Integrated with hatchery | Integrated without hatchery | Independent | Breeder | Future breeder |
| Indoor broiler | Integrated with hatchery | 1.2 [0.5 - 3] | 0.8[0.3 - 2] | 1.0 [0.4 - 2.5] | 0.2 [0.1 - 0.4] | 0.2 [0.1 - 0.4] | 0.3 [0.1 - 0.6] | 0.007 [0.0009 - 0.04] | 0.005 [0.0005 - 0.04] |
| | Integrated without hatchery | 0.8 [0.3 - 2] | 1.2 [0.5 - 3.1] | 1.0 [0.4 - 2.5] | 0.2 [0.1 - 0.5] | 0.2 [0.1 - 0.5] | 0.3 [0.1 - 0.6] | 0.007 [0.0009 - 0.04] | 0.005 [0.0005 - 0.04] |
| | Independent | 0.6 [0.2 - 1.4] | 0.6 [0.2 - 1.4] | 1.2 [0.5 - 2.8] | 0.2 [0.1 - 0.5] | 0.3 [0.1 - 0.5] | 0.3 [0. 2 - 0.7] | 0.008 [0.001 - 0.05] | 0.006 [0.0006 - 0.04] |
| | Integrated with hatchery | 0.6 [0.2 - 1.5] | 0.5 [0.2 - 1.5] | 1.0 [0.4 - 2.4] | 0.4 [0.2 - 0.8] | 0.3 [0.1 - 0.6] | 0.4 [0.2 - 0.7] | 0.008 [0.001 - 0.05] | 0.006 [0.0006 - 0.04] |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Free-range broiler | Integrated without hatchery | 0.5 [0.2 - 1.5] | 0.5 [0.2 - 1.5] | 1.0 [0.4 - 2.4] | 0.3 [0.1 - 0.6] | 0.4 [0.2 - 0.8] | 0.4 [0.2 - 0.7] | 0.007 [0.001 - 0.04] | 0.006 [0.0006 - 0.04] |
| | Independent | 0.6 [0.3 - 1.7] | 0.7 [0.3 - 1.7] | 1.2 [0.5 - 2.8] | 0.3 [0.1 - 0.6] | 0.3 [0.1 - 0.6] | 0.5 [0.2 - 1] | 0.009 [0.001 - 0.05] | 0.007 [0.0007 - 0.05] |
| Selection | Breeder | 0.05 [0.009 - 0.2] | 0.05 [0.009 - 0.2] | 0.07 [0.02 - 0.3] | 0.01 [0.003 - 0.04] | 0.01 [0.003 - 0.04] | 0.02 [0.005 - 0.05] | 0.006 [0.0007 - 0.04] | 0.004 [0.0004 - 0.03] |
| | Future breeder | 0.04 [0.008 - 0.2] | 0.04 [0.008 - 0.2] | 0.06 [0.01 - 0.3] | 0.01 [0.002 - 0.04] | 0.01 [0.003 - 0.04] | 0.01 [0.004 - 0.04] | 0.003 [0.0003 - 0.02] | 0.006 [0.0006 - 0.04] |

Table 5: Daily transmission probabilities of HPAI H5 virus between an infected source farm and an exposed farm (free of infection) according to the type of farms in the French layer production network (median, Q25 and Q75 values of 10 000 iterations). Percentage of transmission probabilities below the transmission probability between independent free-range layer farms: Dark brown: 50-60%, brown: 60-75%, medium brown: 75-85%, light brown: 85-95%, no colour: over 95%

| **Values in %** | | **Exposed farm (free of infection)** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pullet | Indoor layer | | Free-range layer | | Selection | |
| **Source farm (infected)** | | | Integrated | Independent | Integrated | Independent | Breeder | Future breeder |
| Pullet | | 0.2 [0.06 - 0.5] | 0.1 [0.05 - 0.3] | 0.1 [0.05 - 0.4] | 0.1 [0.05 - 0.3] | 0.1 [0.04 - 0.3] | 0.007 [0.001 - 0.03] | 0.005 [0.0007 - 0.02] |
| Indoor layer | Integrated | 0.1 [0.04 - 0.4] | 0.2 [0.09 - 0.6] | 0.2 [0.08 - 0.5] | 0.2 [0.09 - 0.6] | 0.2 [0.09 - 0.6] | 0.007 [0.001 - 0.03] | 0.005 [0.0008 - 0.02] |
| | Independent | 0.1 [0.04 - 0.4] | 0.2 [0.07 - 0.5] | 0.3 [0.1 - 0.7] | 0.2 [0.09 - 0.6] | 0.2 [0.09 - 0.6] | 0.007 [0.001 - 0.03] | 0.005 [0.0008 - 0.02] |
| Free-range layer | Integrated | 0.2 [0.06 - 0.6] | 0.3 [0.1 - 0.8] | 0.4 [0.1 - 0.9] | 0.4 [0.2 - 1] | 0.4 [0.2 - 0.9] | 0.009 [0.002 - 0.04] | 0.006 [0.001 - 0.03] |
| | Independent | 0.2 [0.06 - 0.6] | 0.3 [0.1 - 0.8] | 0.4 [0.1 - 0.9] | 0.3 [0.1 - 0.8] | 0.4 [0.2 - 1] | 0.009 [0.00187 - 0.04] | 0.006 [0.0011 - 0.03] |
| Selection | Breeder | 0.005 [0.0008 - 0.02] | 0.009 [0.002 - 0.03] | 0.009 [0.002 - 0.03] | 0.008 [0.002 - 0.03] | 0.008 [0.002 - 0.03] | 0.006 [0.0008 - 0.04] | 0.004 [0.0004 - 0.02] |
| | Future breeder | 0.004 [0.0007 - 0.02] | 0.007 [0.002 - 0.03] | 0.007 [0.002 - 0.03] | 0.006 [0.002 - 0.02] | 0.006 [0.002 - 0.02] | 0.004 [0.0004 - 0.03] | 0.004 [0.0005 - 0.03] |

## Sensitivity analysis

The sensitivity analysis was conducted on two types of farms: one with the highest transmission probability (integrated indoor broiler farm) (

Figure 2 and Figure 3) and one with the lowest (future breeder farm for broiler production) as source (infected) and exposed (free) farms (Supplementary file 3).

The distribution of transmission probabilities for all pathways in both cases highlighted the importance of the farm biosecurity level on transmission probability. Indeed, it showed that the transmission probabilities are high for all pathways when biosecurity levels of both source and exposed farms are low, but have a lower impact when the biosecurity levels are high (Figure 2).

The biosecurity level of the source farm has less influence on the probability of transmission than the biosecurity level of the exposed farm on the transmission probability, as showed by the distribution of transmission probabilities. For all pathways, the variation in the biosecurity level of the source farm (infected) had a low impact on the variation of the transmission probabilities (Figure 3 A). On the other hand, the variation in the biosecurity level of the exposed farm had a significant impact on the variation in transmission probabilities (Figure 3 B). Indeed, transmission probabilities were high in exposed farms with biosecurity levels below (3 on 5), while they were very low in farms with high biosecurity level (above 4), for all pathways except person movement for both farm types and material movement for future breeder farms. The distributions of transmission probabilities were very similar between the two types of farms for all pathways except for the shared material. As their respective total transmission probabilities were different, this result reinforces the impact of biosecurity level on the transmission.

Figure 2: Distribution of the transmission probabilities across all **pathways** according to the biosecurity level of the source farm (x axis) and to the biosecurity level of the exposed farm (colours) with integrated indoor broiler farm as exposed and source farms. Threshold at 1/100 (black), 1/1000 (red) and 1/10 000 (orange).

Figure 3: Distribution of the transmission probabilities (A) across all **biosecurity levels of the exposed farm (free)** according to the pathways (x axis) and to the biosecurity level of the source farm (infected) (colours) and (B) across all **biosecurity level of the source farm (infected)** according to the pathways (x axis) and to the biosecurity level of the exposed farm (free) (colours) with integrated indoor broiler farm as exposed and source farms. Threshold at 1/100 (black), 1/1000 (red) and 1/10 000 (orange).

## Farm biosecurity and pathways in the broiler sector

The majority of integrated broiler farms (indoor and free-range) were scored with a good biosecurity level (3 in 5) (53% of indoor and 45% of free range) (Supplementary file 4**)**. Independent indoor broiler farms were scored with a higher biosecurity level (43% at good level) than independent free-range broiler farms (42% at low level). For both indoor and free-range farms, independent broiler farms were estimated to have a lower biosecurity level than integrated farms. The pathways which generated the highest transmission probabilities between two integrated indoor broiler farms with a good biosecurity level are person movement, feed transport and manure management (respective median value of 0.23%, 0.03% and 0.03%). The most frequent transmission pathways for integrated broiler farms were person movement (median

occurrence frequency of 21%) and feed transport (median occurrence frequency of 30%). When considering an integrated indoor broiler farm as an exposed farm, the probability of contact through person and feed movement was high if the source farm was an indoor broiler farm integrated in the same farmers' association, medium if the source farm was an indoor broiler farm integrated in another farmers' association and low if the source farm was an independent indoor broiler farm, a free range broiler farm or a selection farm (Figure 4).

The production farms with the lowest transmission probability were integrated free-range broiler farms as exposed farm even if their biosecurity levels were equivalent to biosecurity levels of integrated indoor broiler farms. The pathways with the highest transmission probability between integrated free-range broiler farm at good biosecurity level were material, feed and manure movement (respectively median value of 0.02% and 0.01%). Person movement had a lower impact on the probability of transmission in free-range farms than in indoor farms. The occurrence frequency of person movement between integrated free-range broiler farms (median value of 62%) was lower than between integrated indoor broiler farms (median value of 78%). For both types of farms (integrated indoor and free-range), transport of birds from hatchery (chicks) or to slaughter and dead bird management were the pathways with the lowest impact on the transmission probabilities. Movement of birds from hatchery and to slaughter had the lowest frequency of occurrence (associated with the production cycle). Dead bird management was considered to have a high frequency of occurrence, but this pathway is the only one that stops in the public area of the farm (Supplementary file 1).

Figure 4: Frequency of pathway occurrence in integrated indoor broiler farm according to the different types of pathways and the different type of farms as pathway source (Broiler I int SA: Indoor farm integrated in the same farmer association, Broiler I int OA: Indoor farm integrated in another farmer association, Broiler I ind: Indoor farm independent, Broiler FR: Free-range broiler farm)

## Farm biosecurity and pathways in the layer sector

Indoor layer farms were estimated to have between very good biosecurity level and good biosecurity level. Free-range farms were estimated to have between good biosecurity level. Pullet farms were considered with a good biosecurity level (3 in 5) (50%) (Supplementary file 4).

Due to their production, layer birds transport occurs at a lower frequency than broiler production. Moreover, the pathway with the highest impact on transmission probabilities was egg transport. When comparing the layer production farm with the highest transmission probability (independent free-range layer farms with a low biosecurity level) and the farm with the lowest transmission probability (integrated indoor layer farms with a good biosecurity level), the pathways with the highest transmission probabilities were for both egg transport, person movement and shared material. Nonetheless, independent free-range farms had higher transmission probabilities than integrated indoor farms (median transmission probability for egg transport of 0.09% and 0.59% respectively). According to the five experts of the layer sector, the transport of eggs was the pathway with the highest frequency of occurrence for both indoor and free-range layer farms (median values of 45% and 30%

respectively). Person movements and shared material were just as frequent as the other pathways but they are both associated with direct contact with birds.

## Farm biosecurity and pathways in selection farms

For both broiler and layer sectors, experts considered the biosecurity levels of selection farms to be high to very high (4 to 5 out of 5), implying very low transmission probabilities, except for person movement. As with future breeder farms (Figure 4), shared material has no impact on transmission probability. If the results for breeder farms in both productions were similar, eggs transport and manure management appeared to have higher transmission probabilities in broiler production than in layer production. If the transmission probabilities are very low at high and very high biosecurity levels, they are closed to those observed in production farm for person movement, feed and eggs transport, and manure management if the biosecurity level of the breeder farms reached a good level (3 in 5).

# Discussion

This study looked at the risk of virus transmission within French production networks, i.e. the risk that a virus released by an infected farm be introduced into a free farm by all possible indirect pathways and for all types of farms described in the network analysis. Most of risk assessment studies conducted on the risk of AI virus introduction into a poultry farm are based on risk factors related to the production network and wild birds [25,26]. Several risk assessment studies have been conducted on the risk of introduction or release of AI viruses in different production types [14,15,18,20]. Few studies looked at the risk of virus transmission between farms depending on farm types

[18,27]. None of them considered the farm integration levels and the risk of virus transmission from one farm type of another (e.g. from an indoor broiler farm to a breeder farm).

Another originality of this study is for the inclusion of biosecurity levels in the risk assessment process. Biosecurity classifications developed for poultry farms generally provide a farm-level biosecurity score [22,28,29]. Few studies have assessed biosecurity level according to the farm type (breeder, broiler or layer) [30] or to the level of farm integration [31,32] yet none have used this classification to assess the risk of disease transmission per farm type. Our description of biosecurity levels was largely based on previous work done in France [7,23] and it was adapted and validated with poultry farm biosecurity specialists in France. The biosecurity criteria were illustrated with a scheme (Supplementary file 1) and the experts expressed no difficulties to understand the grid and the biosecurity classification. This approach based on farm biosecurity enabled to study a large number of farm types. The risk assessment conducted on biosecurity level can be used to assess the risk of virus transmission between farms in any other French poultry production network. Likewise, the developed biosecurity grid can be used to assess the biosecurity level of any type of French poultry farm.

The farm biosecurity level of the exposed farm (free of infection) has a major impact on the AI virus between-farm transmission risk regardless the pathway and the biosecurity level of the source farm (infected). The importance of farm biosecurity on AI virus introduction risk has been highlighted in several studies [33–36]. In this study, farms dedicated to reproductive birds (future breeder and breeder farms) were mainly scored 5, indicating very high biosecurity, while production farms scored 3, indicating good biosecurity. Less than 5% of the production farms were considered to be at a

very high biosecurity level. A study conducted in New Zealand found a similar result [28]. Another study in South Korea that measured the risk of HPAI for farms linked by livestock vehicle movement showed a low risk for 73% of breeder chicken farms [18]. Both free-range and indoor integrated broiler farms were considered with an overall good biosecurity level. In contrast, indoor layer farms were considered having a higher biosecurity (level 3 (good) to level 4 (high)) than free-range layer farms (level 2 (low) to level 3 (good)). As biosecurity measures to limit contact between domestic birds and wild birds were included in the biosecurity grid to assess the farm biosecurity level, the impact of free-range was considered in this assessment. This result is interesting as risk assessment studies in other countries have shown that free-range farms are more at risk of virus introduction by wild birds [26,37]. A serological study conducted in France showed that free-range farms were less infected by HPAI than indoor farms, and that biosecurity measures applied in these farms contributed to reduce the risk of virus introduction and/or exposure [38]. Wild birds are known to have a role in HPAI virus introduction and long-distance spread but their role in short distance dissemination is limited [39,40]. The proportion of broiler farms with a low biosecurity level (level 2) was higher in independent farms (40% of indoor and 42% of free-range) that in integrated farms (15% of indoor and 22% of free-range). This result is difficult to compare with other studies because these studies were conducted in countries with very different production context (Indonesia and Thailand) [31,32]. Moreover, the difference observed must be considered carefully as expert confidence was higher for integrated farms (mean of 4.2 and 3.9 out of 5 for indoor and free-range farms respectively) than for independent farms (mean of 3 and 3.4 out of 5 for indoor and free-range farms respectively). Similar results were observed for independent free-range layer farms but not for independent indoor layer farms for which the proportion

of farms with high biosecurity level (mean value of 43%) was higher than the proportion of with good biosecurity level (mean value of 37%). Nonetheless, only 3 experts out of 5 provided data for this type of farms (mean confidence of 3.7 out of 5) which illustrated the lack of knowledge for this minority farm type. This work is based on expert opinion and highlight the need for more detailed data on farm biosecurity. A study carried out in France provided information on biosecurity as a function of farm type (including broiler and layer) and location, but did not investigate independent farms [41]. Moreover, this biosecurity level assessment has been conducted at the national level and do not consider farm spatial heterogeneity in France.

This risk assessment study aimed to investigate all the links between all farm types in the French broiler and layer production networks and thereby identify the type of farms and links associated with the highest AI transmission risk. The links studied only concerned indirect transmission pathways (i.e., humans or fomite) and not direct transmission pathways (i.e., live domestic birds). Live domestic bird movement is known to present high risk of virus transmission, especially in the duck sector where live duck movement occurs between different growing stages [8]. Nonetheless, this pathway is inexistant in broiler farms or at very low frequency in layer and breeder farms (about once every 12-13 months). This is the reason why this study focused on indirect contact which are much more likely to occur during an AI epidemic. In the current study, we considered that fomite (i.e. person, truck or material) management outside of farms was similar for all types of farms and was not sufficient to affect the viral transmission. The disinfection of fomites inside farms is included in the biosecurity classification. If the impact of fomite disinfection outside of farms is the same for all types of farms, this impact will not change the relative risk. Nonetheless, a good cleaning and disinfection process at the truck level would considerably reduce the

transmission risk between farms. Studies conducted in France detected the presence of the viral genome in half (in 2017) and in a third (in 2021) of trucks tested after disinfection [42,43].

Person movement was the pathway with the highest transmission probabilities. This pathway is associated with direct contact with birds and high occurrence frequency. The other pathways including direct contact with potentially infectious substances such as manure or dead birds or shared material in direct contact with birds were associated with high transmission probabilities. Previous works obtained similar results [11,15,20]. Feed transport was also associated with high transmission probabilities. Our study focused on the transmission pathways described as the most at risk of transmission in the literature [10]. Nonetheless, other pathways such as transmission by wind or vermin (i.e. mechanic vectors such as rodents or insects) are also studied in the literature. Air-borne transmission was found to have a limited role in AI viruses transmission in France [44], even though this transmission is possible at short distance as some virus genome was found in air samples of infected farms [45]. Dust seems to have also a role in viral dispersal [46]. Virus transmission through vermin movements between farms is possible but difficult to distinguish from other environmental factors [25,27,47]. These last pathways can be included in an environmental transmission pathway, which is mainly due to high proximity between farms. High poultry density is known to have an impact in the spread of AI viruses [48]. Our study compared the relative transmission risk from one farm to another and was conducted under the hypothesis that environmental transmission pathway will occur in the same way for all types of farms regardless their biosecurity level. Environmental transmission could have an impact on short distance spread and should be considered for studies integrating geographical information, which is not the case for the current work.

In the broiler sector, the transmission risk was higher when exposed farms (free) were indoor farms compared to other type of farms. This is mainly due to the higher frequency that a pathway occurs in this type of farm compared to free-range farms, as previously identified [30]. This transmission risk was the highest between broiler farms integrated within the same farmers' association. The probability of contact through a given pathway was the highest between these farms, especially for person in direct contact with birds and feed truck pathways. This result can be easily explained by the integration structure in France. Indeed, poultry farmers' associations are based on a feed manufacturer and technicians and veterinarians are often employed by this farmer association. The role of the integration of in-between farm spread of AI was highlighted in previous studies [11,13,16]. Contact frequency was higher between farms of the same type (i.e. indoor, free-range and breeder) than between farms of different types. According to experts, fewer contacts occurred between integrated and independent farms, especially for indoor broiler farms. In the layer sector, the transmission probability was higher for free-range layer farms as source (infected), related to the farm biosecurity level. This result confirmed that egg transport have a major role in the transmission probability [18,27]. Even if breeder farms have a very high biosecurity level, the high frequency of pathways increase the risk of virus transmission between breeder farms. Indeed, our result showed that if the biosecurity level of a breeder farm as exposed farm is reduced to a good biosecurity level, the transmission probability is similar to the one for production farms. This transmission risk in breeder farms is confirmed by previous studies [30,33]. The study of HPAI H5 outbreaks between 2020 and 2022 in France showed that at least 9% of outbreaks occurred in a breeder farm [2].

This work provides a full picture of the risk of AI virus spread within French broiler and layer production networks considering the virus transmission risk between any farm types. To mitigate this risk, management measures should focus on biosecurity, especially for the movements that enter the farm. A careful attention should be paid to movement with a high occurrence frequency such as person movement, feed, manure and dead bird transport. Integrator should be aware of the increased risk between farm within the same integration and take actions, such as cleaning and disinfection process between farms, to reduce this risk. This work provides a typology of farms based on the transmission risk which will be useful for the implementation of control measures such as movement restriction or vaccination.

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The authors declare that they have no financial or non-financial conflicts of interest in relation to the content of the article.

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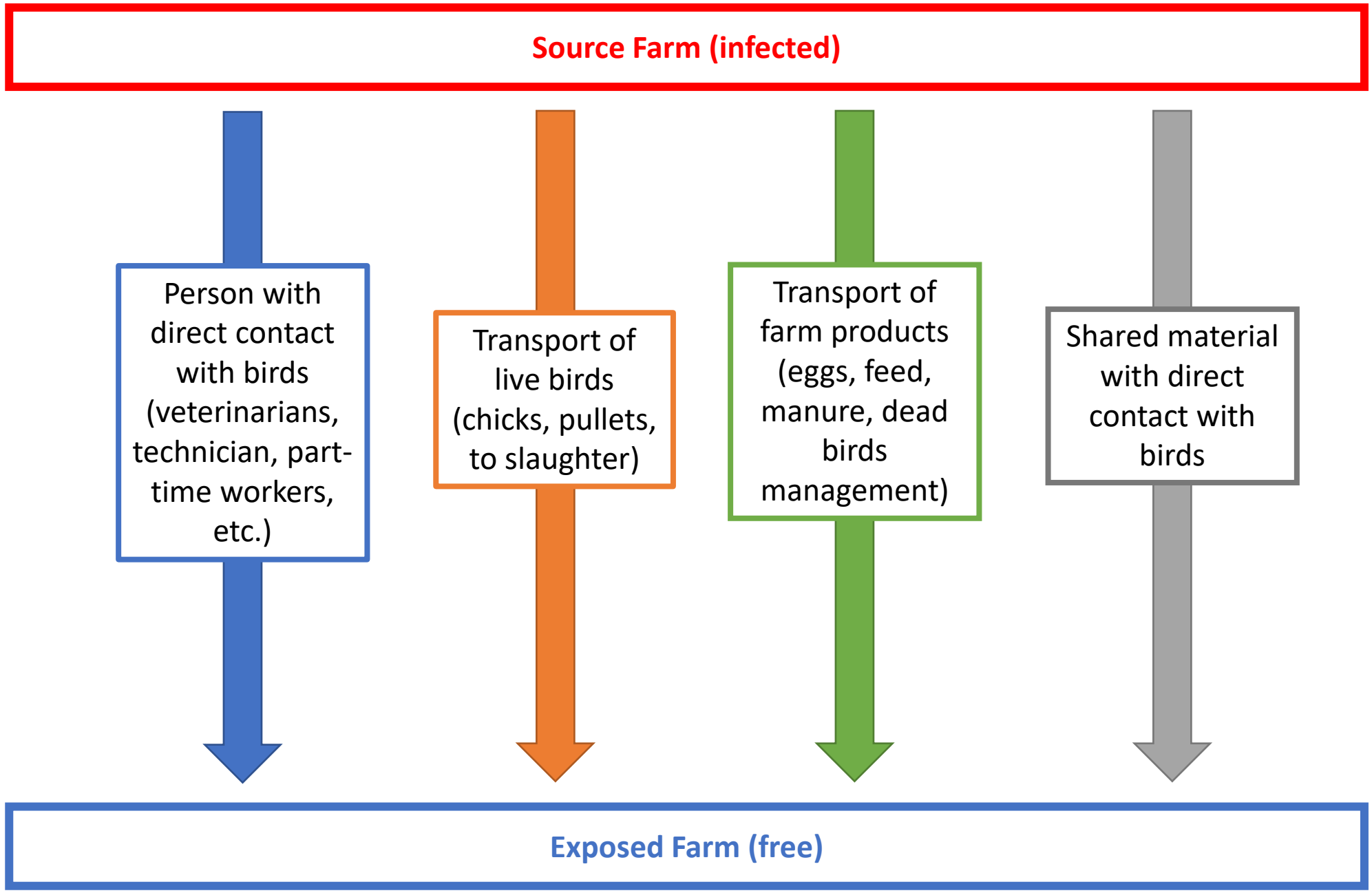
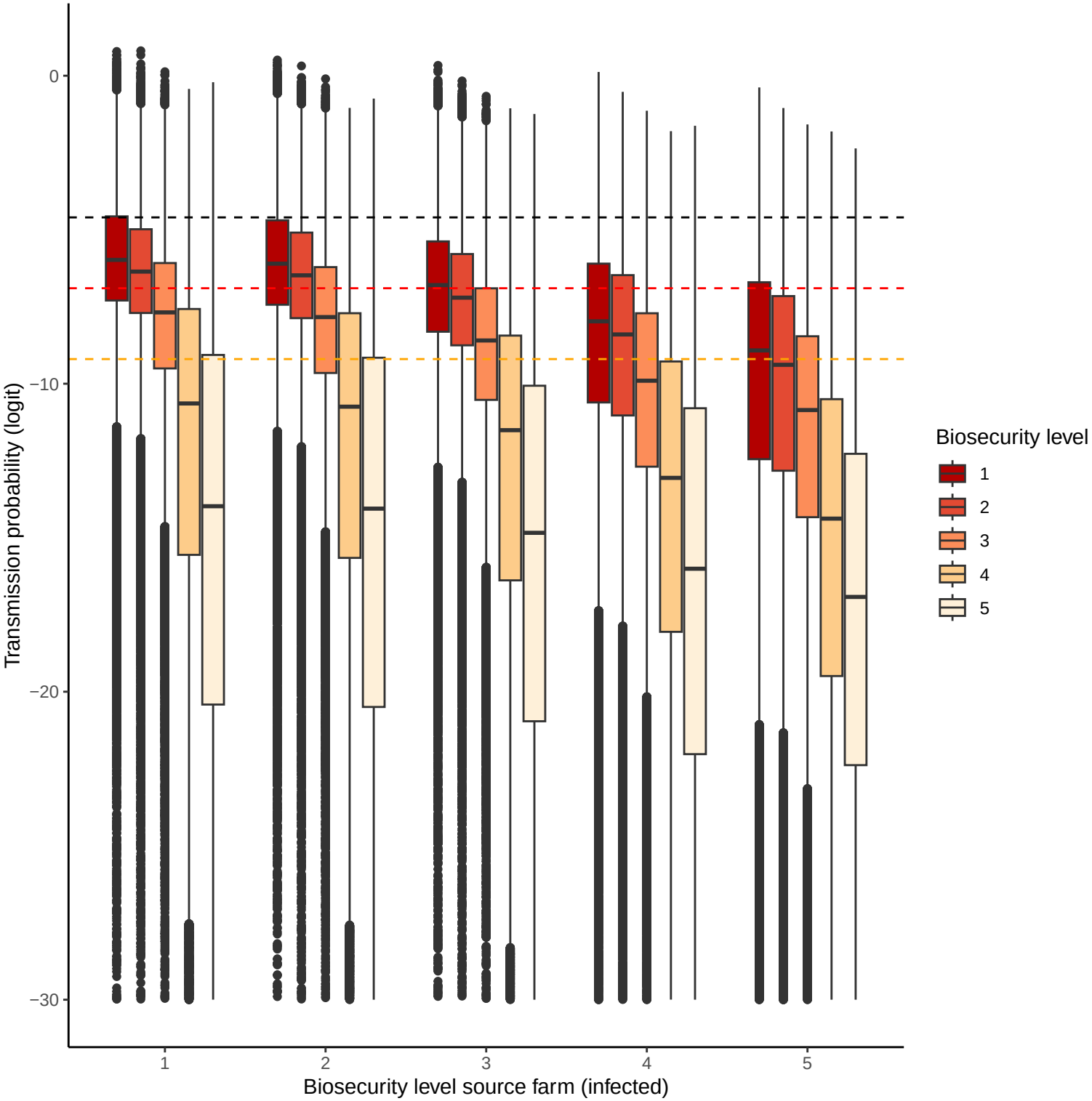
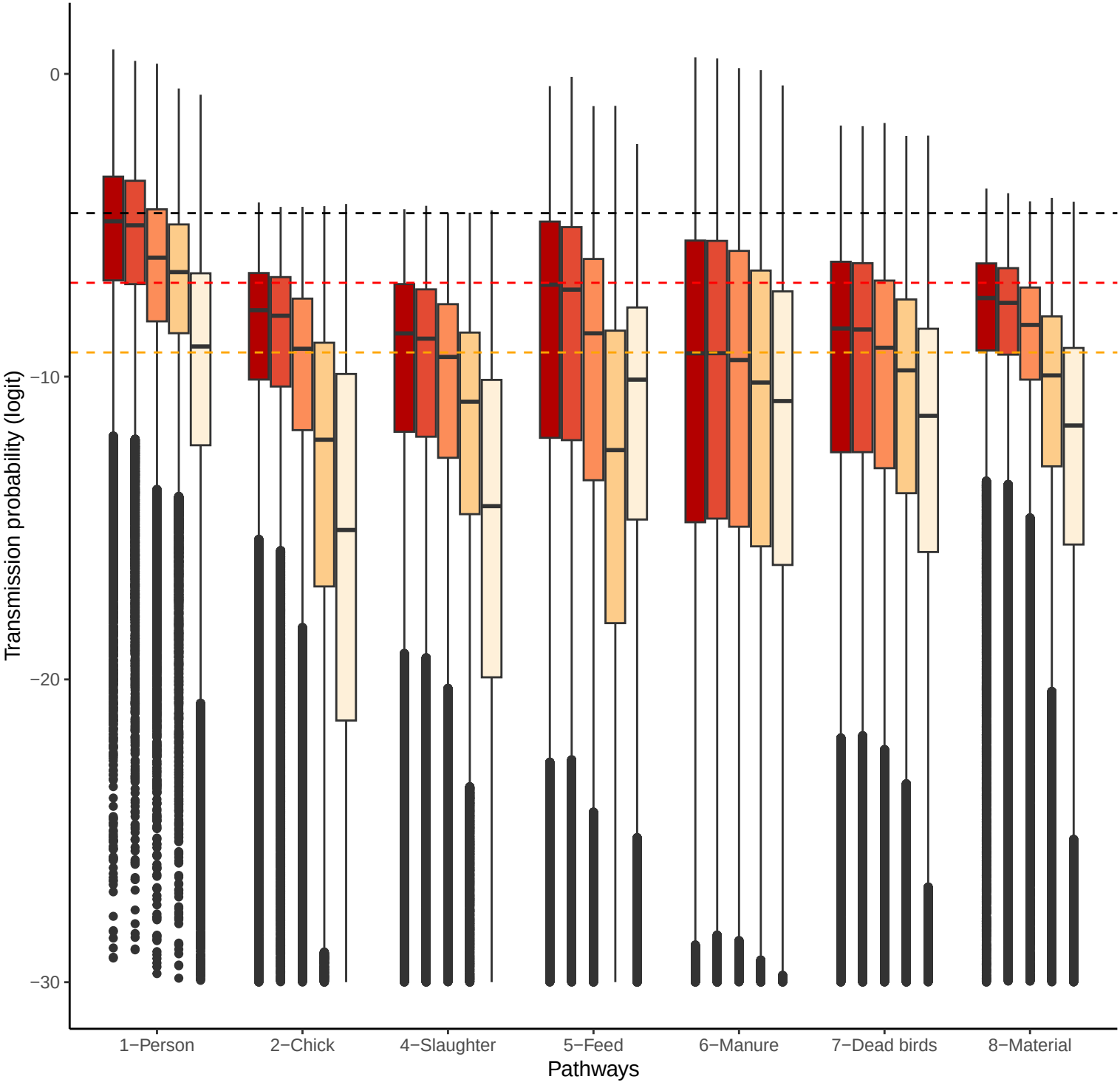
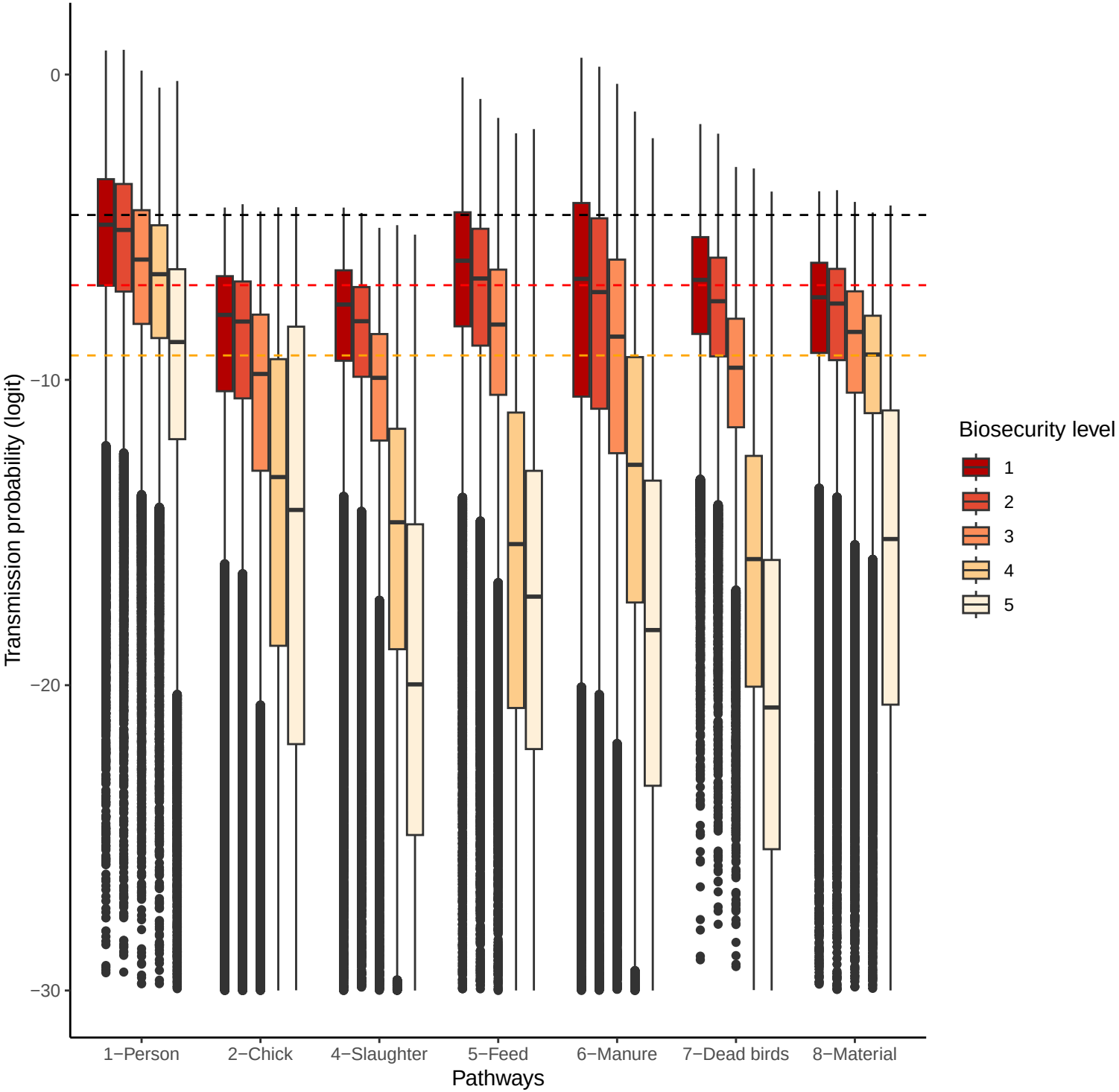

Source Farm (infected)
Person with direct contact with birds (veterinarians, technician, part-time workers, etc.)
Transport of live birds (chicks, pullets, to slaughter)
Transport of farm products (eggs, feed, manure, dead birds management)
Shared material with direct contact with birds
Exposed Farm (free)

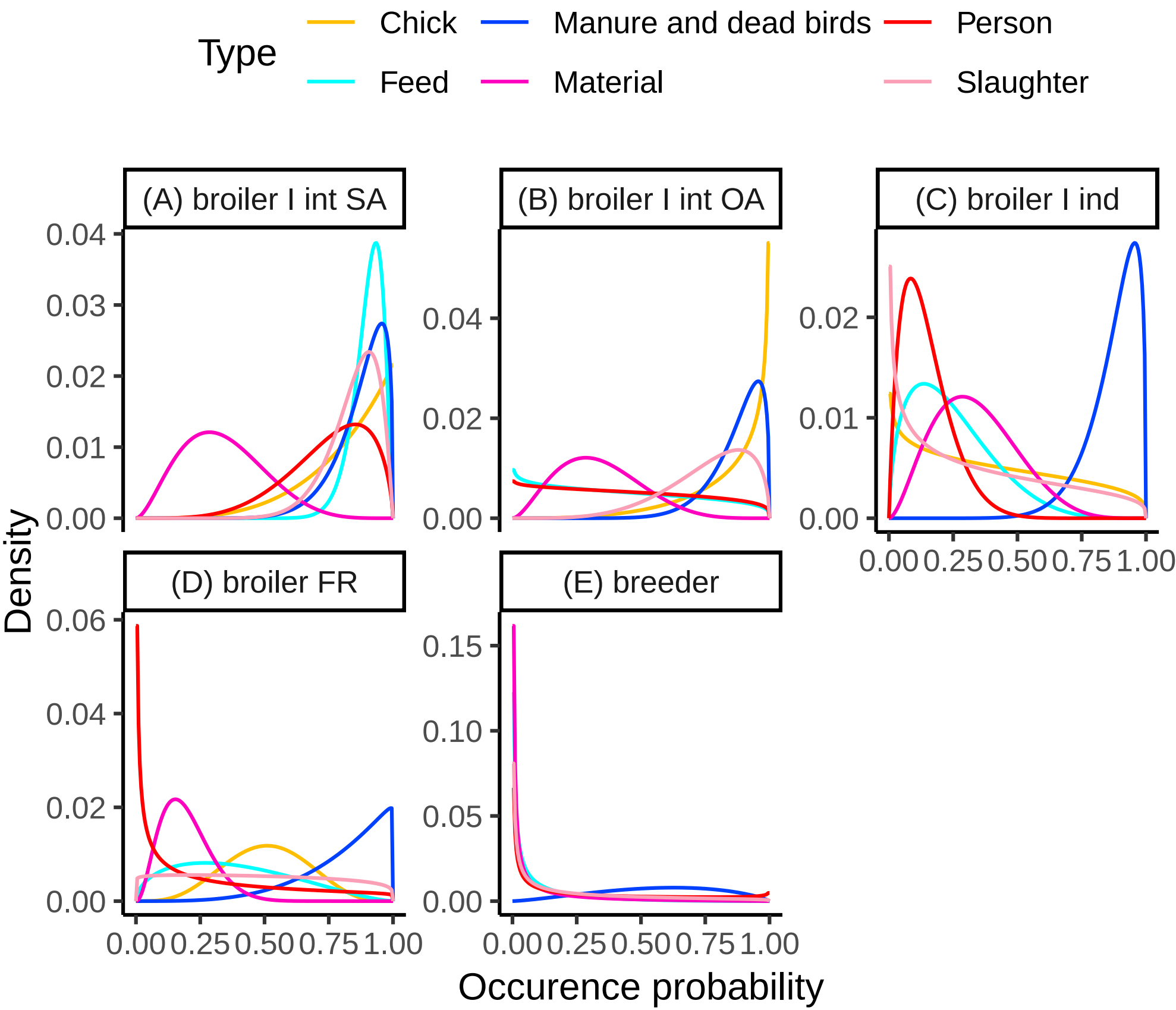

0
−10
−20
−30
Transmission probability (logit)
1
2
3
4
5
Biosecurity level source farm (infected)
Biosecurity level
1
2
3
4
5

(A) Variation on biosecurity levels of the exposed farm (free)



(B) Variation on biosecurity levels of the source farm (infected)




Type
Chick
Manure and dead birds
Person
Feed
Material
Slaughter
(A) broiler I int SA
(B) broiler I int OA
(C) broiler I ind
(D) broiler FR
(E) breeder
Density
Occurence probability
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.10
0.15
0.25
0.50
0.75
1.00