MAJOR ARTICLE

High Probability of Avian Influenza Virus (H7N7) Transmission from Poultry to Humans Active in Disease Control on Infected Farms

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An epizootic of avian influenza (H7N7) caused a large number of human infections in The Netherlands in 2003. We used data from this epizootic to estimate infection probabilities for persons involved in disease control on infected farms. Analyses were based on databases containing information on the infected farms, person-visits to these farms, and exposure variables (number of birds present, housing type, poultry type, depopulation method, period during epizootic). Case definition was based on self-reported conjunctivitis and positive response to hemagglutination inhibition assay. A high infection probability was associated with clinical inspection of poultry in the area surrounding infected flocks (7.6%; 95% confidence interval [CI], 1.4%–18.9%) and active culling during depopulation (6.2%; 95% CI, 3.7%–9.6%). Low probabilities were estimated for management of biosecurity (0.0%; 95% CI, 0.0%–1.0%) and cleaning assistance during depopulation (0.0%; 95% CI, 0.0%–9.2%). No significant association was observed between the probability of infection and the exposure variables.

Since 1997, >400 persons have been reported to be infected with H5N1 highly pathogenic avian influenza (HPAI) virus, including >200 fatal cases [1–5]. Infections of avian influenza viruses in humans are considered a public health risk, because a nonhuman influenza virus might change through genetic reassortment or mutation into a virus capable of efficient spreading among humans, eventually causing a human pandemic [6]. Consequently, it is important to better understand and quantify the risk of humans contracting infections from the avian reservoir [7].

In Europe several avian influenza epizootics have occurred in poultry during the last decade [8]. Prominent examples are the HPAI H7N1 epizootic in Italy in 1999–2000 and the HPAI H7N7 epizootic in The Netherlands in 2003 [9, 10]. During and shortly after the Italian epizootic, 759 serum samples were collected from persons who had been in contact with infected birds. No symptoms were reported, and none of the samples tested positive for H7 antibodies in either cell culture microneutralization or single radial hemolysis tests [11]. A follow-up study after a subsequent epizootic of low-pathogenicity H7N3 avian influenza found 3.8% of involved workers to be positive by a combination of serological test methods [12]. In The Netherlands, >450 persons involved in control activities of the above

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mentioned H7N7 epizootic reported health complaints, particularly conjunctivitis, and 89 persons tested positive for the presence of influenza virus in ocular or throat swab samples by reverse-transcription polymerase chain reaction or culture, as a result of active case finding [13]. Moreover, 1 infected veterinarian died after developing acute respiratory distress syndrome [13–15]. Fortunately, the virus did not seem able to spread efficiently among humans [16]. In the infected persons, no antibodies were detected by the standard methods, but a high proportion (89%) of known infected persons tested positive in a modified hemagglutination inhibition (HI) assay [17]. Similar observations during more recent outbreaks confirmed that serological responses to H7 subtype avian influenza viruses in humans may not be detectable with standard methods [18, 19].

Direct handling of sick and dead poultry and the presence of sick or dead birds in the household were determined to be risk factors for infection with HPAI H5N1 virus [20–22]. Knowledge of the probability of virus transmission from poultry to humans involved in the control of infection on infected poultry farms and risk factors for transmission is important, because it might help identify more adequate measures to prevent infection of humans on an infected poultry farm. However, to the best of our knowledge, this has not yet been done.

Our study aims to fill this gap. It uses the unique data from the Dutch H7N7 epizootic of 2003, which includes information on both the human and the veterinary side of a complete and contained epizootic. The veterinary data have been used to quantify the within-flock transmission of avian influenza virus and develop a simulation model, thus making it possible to quantify the infectious period of an infected farm [23, 24]. The human data registered the farm visits of persons involved in containing the epizootic as well as self-reported disease symptoms and the results of antibody tests for HPAI H7N7 virus [13, 17]. We combined these databases to estimate the probability of transmission of HPAI H7N7 virus from poultry to humans involved in the control of the Dutch epizootic in 2003. We also assessed the association between human infection and factors possibly related to exposure to virus on poultry farms.

MATERIALS AND METHODS

Data on persons involved in control activities. The data on persons involved in control activities on infected farms originated from 2 separate data sets: a database with details of the farm visits and the results of a health impact assessment (questionnaire) conducted by the National Institute of Public Health and the Environment during and after the epizootic. Persons involved in veterinary control activities during the epizootic were registered in various governmental databases, which kept track of the following: the farm visited, date of the visit, and activities performed during that visit. Because no registration system was in place when the epizootic started, it took some time to develop one; hence, the data set does not contain all visits that actually took place. In this study we assume that the risk of infection during the missing visits was similar to that for the recorded visits. The database contained data on 5051 persons, of whom 3165 had visited $\geqslant 1$ of the 241 infected farms where virus was isolated (Figure 1).

Activities were classified as screening, indexing, tracing, or depopulation. Persons involved in depopulation were divided into 3 categories based on probable contact with infected birds: cleaning assistance during depopulation, management of biosecurity during depopulation, and active culling during depopulation (Table 1). In some cases, screening and culling took place on the same date.

Conjunctivitis was a common symptom for HPAI H7N7 infection in humans [13], and a case register was kept during the epizootic. Unfortunately, because of privacy laws in the Netherlands (lack of informed consent) it was not possible to link the case register with our database. However, during and after the epizootic a questionnaire was sent to persons involved in control of the epizootic and possibly exposed to infected poultry to ask whether they had eye complaints after 1 March 2003. If they answered yes, they were asked to note the period during which they had eye complaints and whether these complaints included burning eyes, red eyes, teary eyes, itching eyes, sensitivity to light, purulent fluid in eyes, painful eyes, or other symptoms [13]. Subjects were classified as having conjunctivitis if they reported $\geqslant 2$ of these symptoms in the questionnaire response. The questionnaire also asked for information on the use of personal protective equipment (PPE; eg, masks and goggles). However, these data were incomplete compared with the other data and were not registered per farm visit; therefore,

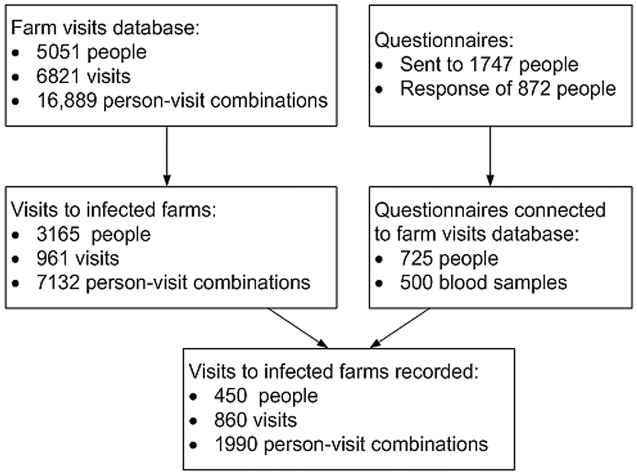

Figure 1. Overview of the origin and structure of the data.

Table 1. Activities of Persons Involved in Control of Infection on Infected Farms

Activity	Definition
Screening	Clinical inspection of poultry in protection and surveillance zone and collecting (blood) samples of birds or complete carcasses to send to laboratory
Indexing	Assessing value of the flock to be culled by estimating the number of dead, diseased, and healthy birds; generally, no entering of poultry house and no physical contact with poultry
Tracing	Clinical inspection of poultry on contact farms and collection of (blood) samples from birds or complete carcasses to send to laboratory
Depopulation	
Cleaning assistance	Assisting in cleaning of equipment used during or around depopulation (eg, shower units); no physical contact with poultry
Management of biosecurity	Managing compliance to biosecurity measures for persons present in and around infected premises; no physical contact with poultry
Active culling	Culling carried out by (1) gassing whole poultry houses with carbon monoxide or carbon dioxide, (2) gassing birds in containers with carbon monoxide or carbon dioxide, (3) electrocution, or (4) injection [25]; active handling of dead poultry (method 2) or live poultry (methods 1, 3, and 4)

they could not be included in this study. We assumed that PPE use was similar among the various categories included in the study.

Participants were also asked to provide blood samples, which were subsequently tested serologically for H7 antibodies by a modified HI assay, using horse erythrocytes and 2 hemagglutinating units of virus [17]. This approach was validated by defining cutoffs based on comparative evaluation of HI titers observed in known infected persons and known uninfected controls. A positive case definition in the present study required both self-reported conjunctivitis and a positive HI assay outcome (cutoff, $\geqslant$10). Sensitivity analyses were also done using these variables individually as readouts.

In total, the questionnaire was sent to 1747 persons (Figure 1), of whom 872 returned the questionnaire and 500 also provided blood samples. Questionnaire response varied between 30% and 73% for different groups of persons classified according to the nature of the activities they performed [26]. The lowest response was found for the group that performed active culling during depopulation. During the epizootic, ~530 persons active in depopulation were from outside The Netherlands, and they could not be included in the health impact assessment.

Farm data. Characteristics of the epizootic in poultry have been described elsewhere [9]. Virus was isolated from 241 commercial farms for which farm characteristics (housing type, poultry type, farm size) and daily mortality data were collected. The mortality data were used to estimate the period during which each farm was infectious. We used the mathematical simulation model of Bos et al [23] and adapted it with parameters described elsewhere [24]. Farms were considered to become infectious when the model predicted that $\geqslant$1 infectious bird was present and were considered to remain infectious until they had been depopulated.

Some commercial farms included >1 infected flock (267 infected flocks on 227 farms); in these cases, the longest possible infectious period was used. For the 38 flocks for which daily mortality was not registered, the start of the infectious period was set at 4 days before depopulation, based on the median of the flock-infectious period for flocks without missing data. For the 52 flocks for which the total number of birds was unknown, the start of the infectious period was also set at 4 days before depopulation.

Linking the data sets. The farm visits database and the questionnaire data were linked by a unique person identifier (Figure 1), and the final human data set contained only the person-records that included both questionnaire data and $\geqslant$1 recorded visit to an infected farm per person. The data sets of infected farms and human cases were linked by the case number of the farm. The final aggregated data set included the following farm data: case number, farm size (number of birds present; smaller or larger than median), poultry type (chicken, turkey, broiler, or other), housing type (battery cages, loose housing, or other), estimated date of infection, date of depopulation, and method of depopulation (gassing, electrocution, or other). The human data in the final data set consisted of person identifier, case number of visited farm, date and time of visit (before or after 14 March 2003, when oseltamivir began being used), goal of visit, status of conjunctivitis, and outcome of HI assay.

Data analysis. The analyses are based on the assumption that each susceptible individual i has a probability p_{ij} to be infected during his/her jth visit to an infectious farm. Hence, the probability that a susceptible individual i is not infected at the jth visit is $1-p_{ij}$, and the probability that an individual i escapes infection altogether is $\prod_{j=1}^{n_i}(1-p_{ij})$, where n_i denotes the total number of farm visits made by individual i. Similarly, an infected individual may have become infected during any single visit, yielding the following overall probability of infection for individual i: $\sum_{j=1}^{n_i} p_{ij}\prod_{k=1}^{j-1}(1-p_{ik})$.

Using the above notational conventions and denoting the total set of individuals by P, the set of infected individuals by

I, and the set of uninfected individuals by $P \setminus I$, the likelihood is calculated as follows:

$$L(\mathbf{p}) = \prod_{i \in P \setminus I} \prod_{j=1}^{n_i} (1 - p_{ij}) * \prod_{i \in I} \sum_{j=1}^{n_i} p_{ij} \prod_{k=1}^{j-1} (1 - p_{ik}) \ , \qquad (1)$$

where $\mathbf{p} = (p_{ij})$ represents the set of parameters.

The model specified by Equation (1) is fully saturated, because it contains a parameter for each person-visit. In the following, we have restricted attention to a number of specific scenarios that contain a much smaller number of parameters. First, a model was used that distinguished between the types of visits described in Table 1. Second, we have classified person-visits according to the exposure variables. In essence, the parameters incorporated in the model (type of visit and exposure variables) are independent covariates, and the case definition is the dependent variable.

The parameters of interest were estimated by maximizing the likelihood function in Equation (1). Confidence intervals [CIs] of the parameter estimates were calculated using the profile likelihood [27]. Within an outcome set specified by the case definition used, models of different complexity were compared by Akaike's information criterion [28]. Likelihood ratio tests were used to assess whether differences in parameter estimates were significant. Potential risk factors, such as PPE use, poultry handling experience, years of poultry exposure, and educational level, were assumed to be similar among the various categories, owing to the lack of data for these factors. Sex ratios were also assumed to be similar among the categories (nearly all persons were male [$n = 399$]).

RESULTS

Descriptive data. Of the 872 persons in the questionnaire data, 725 could be linked to the farm visits database (Figure 1). Of these, 450 persons could be linked to ⩾1 visit to an infected farm, representing 860 visits and 1990 person-visits to infected farms. The remaining 275 persons had no recorded visit to an infected farm. Fifty-eight persons had self-reported conjunctivitis, 159 had a positive HI test, and 26 had both and were therefore defined as case patients.

Table 2 shows the number of observations per category. Notice that a single person could make multiple person-visits and perform multiple activities. Most person-visits were for depopulation ($n = 611$), and 43 of 296 person-visits for active culling involved case patients, compared with 13 of 295 person-visits for biosecurity management. Person-visits for tracing were the second-largest group, with 73 person-visits from case patients among 235 total person-visits in this category. Indexing and screening yielded 5 and 7 person-visits from case patients among totals of 211 and 46 person-visits, respectively. No person-visits for cleaning involved case patients.

Table 2. Descriptive Data for Persons, Person-Visits, and Farms Included for Each Category in the Database

Data	Persons[a]	Person-visits[b]	No. of farms
Screening	5/34	7/46	29
Indexing	2/41	5/211	162
Tracing	3/13	73/235	148
Depopulation			
Cleaning assistance	0/17	0/20	14
Management of biosecurity	2/95	13/295	165
Active culling	19/130	43/296	163
Depopulation method			
Gassing	17/119	33/240	127
Electrocution	8/36	8/50	32
Other	2/6	2/6	4
Housing type			
Battery cages	10/65	18/93	40
Loose housing	14/100	20/184	109
Unknown or combination	5/16	5/19	14
Poultry type			
Turkeys	1/25	1/29	15
Chickens	19/122	42/265	146
Broilers	0/2	0/2	2
Date of visit			
Before 14 March 2003	10/50	13/74	42
After 14 March 2003	15/109	30/222	123
Farm size			
⩽8800 birds	10/80	14/126	71
>8800 birds	13/90	23/134	71
Unknown	4/26	6/36	21

[a] Data represent number of persons with defined influenza cases ($n = 26$)/total number of persons ($n = 450$). One person could perform multiple visits and multiple activities.

[b] Data represent number of person-visits performed by persons with defined influenza cases/total number of person-visits.

Most farms in the database were depopulated by gassing (127 of the 163 farms), and loose housing was more common than battery cages (109 vs 40 farms). Adult chickens were the main poultry type (146 farms vs 15 turkey farms and 2 broiler farms). Most farms were depopulated after the first 2 weeks of the epizootic (123 of 165 farms), and the median of number of birds present was 8800.

Model results. Table 3 shows the estimated probabilities of transmission of H7N7 virus from poultry to humans during a visit to an infectious farm. The highest estimated probabilities of infection were for screening visits (7.6%; 95% CI, 1.4%–18.9%) and active culling (6.2%; 95% CI, 3.7%–9.6%). The lowest estimated probabilities were for cleaning assistance during depopulation (0.0%; 95% CI, 0.0%–9.2%), management of biosecurity during depopulation (0.0%; 95% CI, 0.0%–1.0%), and indexing (0.5%; 95% CI, 0.0%–2.2%). For tracing, the probability of infection was 1.5% (95% CI, 0.3%–4.0%). The probabilities of infection during visits for indexing or biosecu-

Table 3. Probability of Transmission of Avian Influenza (H7N7) Virus from Poultry to Humans during Visits to Infected Farms, by Activity

Activity	Probability, % (95% CI)
Screening	7.6 (1.4–18.9)
Indexing	0.5 (0.0–2.2)[a]
Tracing	1.5 (0.3–4.0)
Depopulation	
Cleaning assistance	0.0 (0.0–9.2)
Management of biosecurity	0.0 (0.0–1.0)[b]
Active culling	6.2 (3.7–9.6)[c]

NOTE. Akaike's information criterion for this analysis was 164.3. CI, confidence interval.

[a] Significantly different from the probability for screening ($P<.05$).

[b] Significantly different from the probabilities for screening ($P<.05$) and tracing ($P\leqslant.05$).

[c] Significantly different from the probabilities for indexing ($P<.001$), tracing ($P\leqslant.05$), and biosecurity ($P<.001$).

rity management were significantly smaller than that for screening visits. Biosecurity management visits had a significantly lower probability of infection than tracing visits. The probability of infection during active culling visits was significantly higher than those for indexing, tracing, and biosecurity management visits. Age and smoking did not have a significant effect on the infection probability (results not shown).

Table 4 shows the transmission probabilities by exposure factor within the category of active culling during depopulation. None of the factors was significantly associated with the probability of infection, although the probability of infection during electrocution seemed higher than during gassing. Furthermore, persons working on infectious farms before 14 March 2003 had a higher probability of infection than those working on infectious farms after this date.

DISCUSSION

The combined analysis of farm data and data from control activities provided a unique opportunity to give quantitative estimates for the transmission of viruses from poultry to humans. Our findings showed that the probability of human infection with avian influenza (H7N7) virus was highest during active culling and screening activities and lowest during cleaning assistance and management of biosecurity during depopulation. We did not observe a significant association between the probability of transmission and the number of birds present on the farm, the poultry type, the housing system, or the depopulation method.

The highest probabilities were found for active culling and screening activities (6.2% and 7.6%, respectively), probably because these activities involve direct handling of (infected) poultry. In England during an avian influenza (H7N3) outbreak, the odds ratio for possible or confirmed cases was as high as 7.5 for working versus not working on an infected farm [19]; in contrast, no significant association between illness reports and exposure to infected birds was found for avian influenza (H7N3) in Canada [29]. The case definition in the Canadian study was based on conjunctivitis only, which may have caused the lack of significant association. However, touching and butchering poultry were also found to be associated with the presence of H5 antibodies in poultry workers in Hong Kong [22].

The finding that cleaning assistance and management of biosecurity during depopulation had low estimated probabilities of infection may reflect the fact that these activities do not involve active handling of poultry. The low probability of infection for indexing (0.5%) may be explained by the fact that most indexers use prior reports written by veterinarians and do not enter poultry sheds. Tracing had a slightly higher probability (1.5%), which can also be explained by contact with possibly ill birds. The possible difference in infection probabilities for tracing and screening visits (1.5% vs 7.6%) is remarkable, because these types of visits involve the same activities. The difference may reflect the fact that persons performing tracing activities are alerted, because the purpose of tracing is to follow up on high-risk contact farms. These persons may therefore take more stringent precautions. If the risk of infec-

Table 4. Probability of Transmission of H7N7 Highly Pathogenic Avian Influenza Virus from Poultry to Humans during Visits to Infected Farms for Active Culling during Depopulation, by Exposure Factor

Exposure factor	Probability, % (95% CI)	Akaike's information criterion
Depopulation method		167.5
Gassing	5.5 (2.7–09.4)	
Electrocution	8.3 (1.0–20.7)	
Other	20.8 (0.0–66.2)	
Housing type		165.1
Battery cages	7.2 (2.1–15.1)	
Loose housing	4.5 (1.8–8.7)	
Unknown or combination	20.1 (4.0–43.8)	
Poultry type		165.0
Turkey	0.0 (0.0–8.1)	
Chicken	7.0 (4.2–10.8)	
Broiler	0.0 (0.0–61.8)	
Date of visit		164.8
Before 14 March 2003	10.2 (3.8–19.6)	
After 14 March 2003	4.8 (2.2–8.8)	
Farm size		167.9
≤8800 birds	5.0 (1.7–10.6)	
>8800 birds	7.5 (3.3–13.6)	
Unknown	5.7 (0.3–18.9)	

NOTE. CI, confidence interval.

tion for one type of visit is relatively low compared with the number of visits made by infected individuals (Table 2), these individuals may have been more likely to become infected during other types of visits.

In the present analyses we did not include PPE use, owing to lack of data. As a consequence, our analyses assumed that PPE use was similar among the categories in the analyses. Another limitation is that we did not adjust for oseltamivir use in the analyses, although this could be a possible confounder. However, we do believe our results to be robust, and we did include a time effect reflecting the date oseltamivir began being used (Table 2). The differences in transmission between the early and late phases of the epidemic may also be partly explained by an increase in PPE use over time.

Of the 450 persons in the database, 26 were positive for infection according to our strict case definition of self-reported conjunctivitis and an HI assay score $\geqslant$10. Sensitivity analyses were performed by using alternative case definitions based on either conjunctivitis or an HI score $\geqslant$10 (results available from M.E.H.B.). The use of conjunctivitis for case definition produced results similar to those in Tables 3 and 4, except for the probability of infection for screening visits, which almost doubled, from 7.6% to 13.0%. Alternatively, when the case definition was based on serological scores, the estimated probabilities of infection increased substantially. This is a direct result of the higher number of cases ($n = 159$) resulting from the same number of visits. We chose to use the stricter definition to increase the specificity, but these findings illustrate the importance of reliable serological methods to assess the true extent of transmission of avian influenza virus to humans.

The infectious periods for the farms were based on the estimated dates of virus introduction, back-calculated from farm mortality data. In this analysis, the farm is considered to become infectious if 1 infectious bird is estimated to be present. Here, the sensitivity analyses indicated that only the transmission probability for screening visits increases to 21.2%; the other probabilities remain similar (results not shown) if a farm is considered to become infectious only when ~1000 birds are infectious. Another sensitivity analysis we performed was on the assigned length of the infectious period for farms with missing data. After we adapted the infectious period from 4 to 6 days for these farms, the probabilities of infection did not change.

Although the data used in this study are unique, like most data sets originating from the field they contain some gaps. During the beginning of the epizootic, no registration systems were in place to record the visits made to farms to control and contain the epizootic. The system had to be developed, and therefore not all farm visits for all persons involved in control of the epizootic were recorded during its early phase. However, even when the registration system was in place during the later stage of the epizootic, recording of farm visits was not perfect because the recording of data was aimed primarily at organizing control efforts. Not all persons responded to the questionnaire, and therefore some could not be included in the final database. In particular, the questionnaire response rates were low for cullers (30%) and destruction and disinfection employees (41%) [26]. The highest response rates were found for government employed (69%) and external veterinarians (73%), groups usually employed in indexing, tracing, screening, and biosecurity management during depopulation activities. The low questionnaire response among the groups with the highest risk (ie, the active cullers) may bias the outcome of the study, but we have no data to support the assumption that the proportion of defined cases in the nonresponding group would differ from that for persons included in this study. We also have no data to suggest that there were age or sex differences between respondents and nonrespondents.

To summarize, the results of this study showed that persons involved in control activities on infected farms have a high probability of infection with avian influenza (H7N7) virus. This is especially true for activities that involve handling of possibly infected poultry, such as clinical inspection of poultry in the area surrounding infected flocks (screening) and active culling during depopulation. Therefore, this study emphasizes the need to take the right protective measures during control activities on infected farms.

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