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# Reproductive ratio for the local spread of highly pathogenic avian influenza in wild bird populations of Europe, 2005–2008

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## SUMMARY

Highly pathogenic avian influenza (HPAI) has devastating consequences for the poultry industry of affected countries. Control of HPAI has been impaired by the role of wildlife species that act as disease reservoirs and as a potential source of infection for domestic populations. The reproductive ratio ($R_0$) of HPAI was quantified in nine clusters of outbreaks detected in wild birds in Europe (2005–2008) for which population data were not available. The median value of $R_0$ was similar (1·1–3·4) for the nine clusters and it was about tenfold smaller than the value estimated for poultry in The Netherlands in 2003. Results presented here will be useful to parameterize models for spread of HPAI in wild birds and to design effective prevention programmes for the European poultry sector. The method is suitable to estimate $R_0$ in the absence of population data, which is a condition typically observed for many wildlife and certain domestic species and systems.

**Key words**: Avian influenza, Europe, reproductive ratio, wild birds.

## INTRODUCTION

Highly pathogenic avian influenza virus (HPAIV) strains have devastating consequences for poultry flocks because of their high transmissibility and high mortality rates [1]. Epidemics of H7 HPAIV that affected the poultry industries in The Netherlands (2003) and Italy (2000) were probably the result of the introduction of H7 low pathogenic avian influenza (AI) strains from wild birds, as suggested by the simultaneous circulation of closely related H7 subtypes in wild birds and poultry [2–4]. Wild birds have also played a major role in the rapid spread of H5N1 HPAIV throughout the world since 2005, which has resulted in >60 countries being affected and the death of millions of domestic poultry and >10 200 wild birds in Central Asia, the Middle East, Europe, and Africa [5, 6]. In an attempt to achieve early detection of future HPAIV incursions, research efforts in Europe were aimed at improving the sensitivity of surveillance measures in wildlife populations, e.g. towards the identification of environmental risk factors and new sentinel species [7, 8] or the evaluation of surveillance systems and sampling efforts [9, 10]. However, those efforts have been impaired by the lack of knowledge on critical aspects of the epidemiology of HPAIV transmission in wild birds that are yet to be

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elucidated. For example, there is a need to understand more completely and in quantifiable terms the mechanisms of virus spread in the field. Knowledge of the mechanisms driving transmission of HPAIV is a prerequisite for the formulation of realistic models for HPAIV spread in wild birds, and it is critical to predicting and preventing epidemics and pandemics of emergent strains of the virus.

The disease reproductive ratio ($R_0$) is a key parameter for understanding disease dynamics. Under specific conditions, values of $R_0 = 1$ indicate that the disease has reached an equilibrium in the susceptible population, so that every infectious individual infects, throughout the duration of the period of infectiousness, one single susceptible individual. For that reason, the disease is likely to persist in the population, which may lead to a state of endemicity. Conversely, values of $R_0 > 1$ suggest that the disease is actively propagating throughout the susceptible population. Finally, values of $R_0 < 1$ indicate that because the number of new infections is on average lower than the number of infectious individuals, the epidemic is dying off [11]. Therefore, estimates of $R_0$ are critical in disease control and for the parameterization of spread and transmission models. Such models may be used to predict geographical areas and periods of time in which the disease is most likely to occur, to estimate the epidemiological and economic impact of a disease, to simulate alternative control strategies, and to quantify the resources required for disease surveillance and control programmes [12].

Techniques commonly used to estimate $R_0$ require knowledge on the size and distribution of the susceptible population, which is typically unknown for many wildlife species. Here, we applied an algorithm that does not make use of population data and that was derived from the expected growth rate of an epidemic to quantify the value of $R_0$ in wildlife species affected by H5N1 HPAIV in Europe from 2005 to 2008. Although this method was previously used to compute the farm-level AI reproductive of ratio for domestic birds [13], to the best of our knowledge, this is the first time that it has been used in wildlife and, moreover, it is also the first time that estimates of the value of $R_0$ for HPAI in wild birds have been published in the peer-reviewed international literature. Results presented here could help to formulate and parameterize spread models for the disease in wild birds and will ultimately contribute to enhance the effectiveness of national and regional HPAI surveillance and control programmes. The method might be easily applied to estimate the value of $R_0$ for other disease epidemics in absence of population data.

## METHODS

Data used for the analyses included the location (latitude, longitude) and the date of identification of the 591 H5N1 HPAI cases in wild birds reported by European countries to the World Animal Health Organization (OIE) from October 2005 to December 2008. Countries reported H5N1 HPAI cases using the definition for outbreaks provided by the OIE. According to the OIE, infectious disease outbreaks are defined as the occurrence of one or more cases of a disease or an infection in an epidemiological unit, where an epidemiological unit is represented by a group of animals with a specific epidemiological relationship that share approximately the same likelihood of exposure to a pathogen [14]. However, when this recommendation is applied to wildlife species, delimitation of epidemiological units becomes, in most of cases, vague or ambiguous. For that reason, some countries may have interpreted more than one case as a single outbreak, whereas others may have combined both concepts into one single metric. To overcome this issue, we selected specific areas within the temporal and geographical extension of the epidemic to conduct specific case-studies for which the value of $R_0$ was computed. Ecological and epidemiological conditions such as season, year, criterion used to report the outbreak, sensitivity of the surveillance system, or species affected, were likely to be different for each selected area. Thus, the variation in the value of $R_0$ estimated here is a proxy for the expected variation of the parameter associated with those factors over the course of the epidemic.

Areas for the case-study were selected and delimited using an algorithm for the identification of time–space clustering. The procedure used to identify the clusters and results of the analysis have been described elsewhere [15]. A scan-based time–space cluster analysis technique was used to limit geographical locations and period of time in which H5N1 observed outbreaks are significantly higher than the expected. Candidate clusters of disease were identified using time–space cylinders that were placed at each location in which outbreaks were reported [16]. The base and the height of the cylinders represented, respectively, the spatial and temporal dimensions of the data. The statistical significance of the identified clusters was evaluated using Monte Carlo simulation. Nine time–space

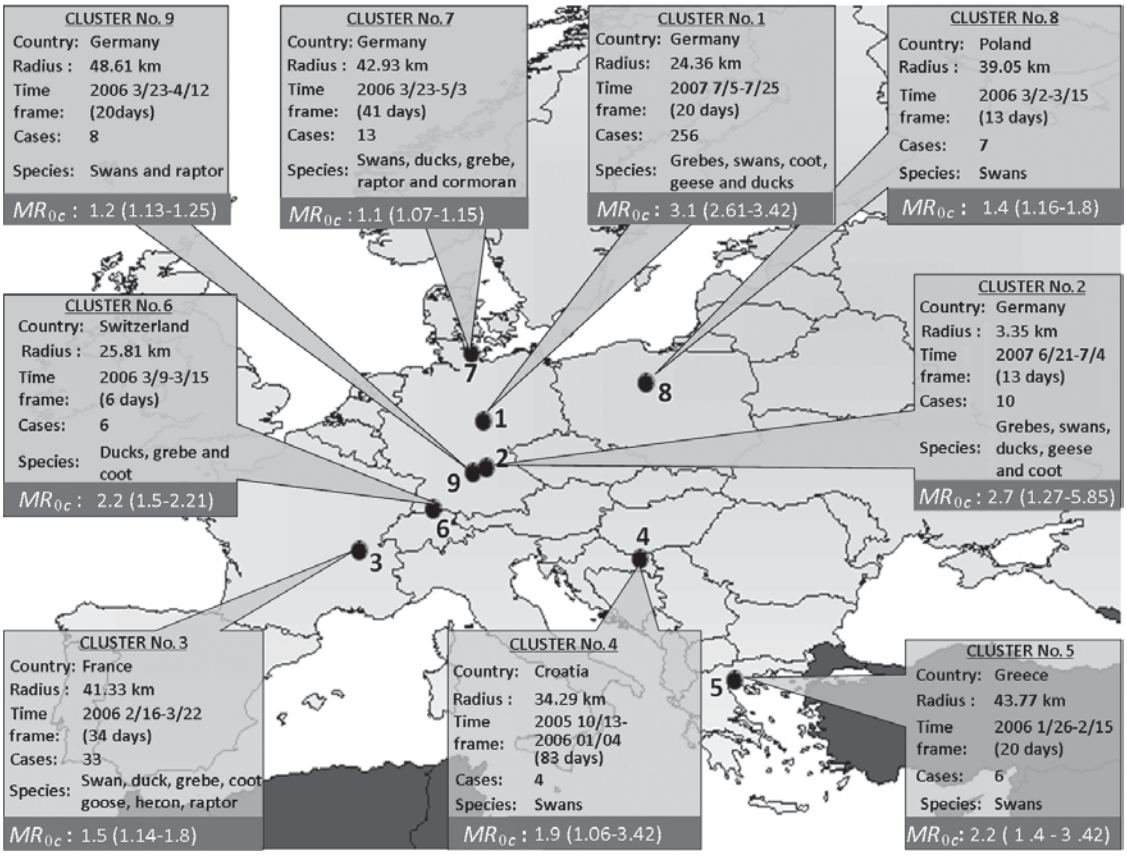


**Fig. 1.** Median (minimum, maximum) reproductive ratio ($MR_{0c}$) of H5N1 highly pathogenic avian influenza virus estimated for nine time–space clusters of cases reported in wild birds in Europe, 2005–2008.

clusters with a temporal duration of >1 day were identified. Clusters were different in terms of number of outbreaks, duration, radius, species affected, season, and year (Fig. 1). For that reason, similar results in the most likely values of $R_0$ were interpreted as evidence of robustness of the estimates to variations in factors such as case-definition, sensitivity of the surveillance system, animal density, or species affected.

Computation of $R_0$ was derived from the growth rate of outbreaks [17] observed within each time–space cluster. Assuming $C_t$ and $C_0$ to be, respectively, the number of cases detected in time $t$ and the number of cases at the beginning of the epidemic, i.e. when $t=0$. The relationship between $C_t$ and $C_0$ is given by

$$C_t = C_0 \exp(\Lambda t),$$

where $\Lambda$ is the rate at which new cases occur and $t$ is time. The relationship between $R_0$ and $\Lambda$ is given by the duration of infectiousness ($D$) [17], so that

$$\Lambda = (R_0 - 1)/D.$$

In lay terms, within each cluster the epidemic grows at an exponential rate in which each case produces $R_0$ new cases during the duration of the infectious period $D$ and, subsequently, the animal does not produce more cases. For that reason $(R_0 - 1)$ new cases are generated during the duration of $D$.

Therefore, $\Lambda$ may be estimated as

$$\Lambda = \ln(C_t/C_0)/t,$$

and subsequently

$$R_0 = 1 + D \ln(C_t/C_0)/t,$$

Considering that it is expected that $C_0 = 1$, i.e. one case, usually referred to as the index case, starts the epidemic, time may be expressed as $t = t_d$, where $t_d$ is the time taken for the number of cases to double, which results in $C_t = 2$. Thus, $R_0$ may be simply computed as

$$R_0 = 1 + (D/t_d) \ln 2.$$

Because the value of $t_d$ can be computed from the intra-cluster epidemic curve, the only assumption required here refers to the value of $D$. Because the true duration of infectiousness of infected animals within each cluster ($D$) was unknown, $D$ was alternatively

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parameterized using most likely, minimum, and maximum values. A value of $D=7$ days, which is an average number assumed by other studies, was also assumed here as the most likely value of the parameter [13, 18] and values of $D=1$ and $D=z$, where $z$ is time to the last case reported in the cluster, were considered the minimum and maximum possible values of the parameter, respectively. Thus, the values of $D=1$ and $D=z$ are equivalent to assuming the extreme case-scenarios in which infected animals remain infectious for 1 day only and throughout the duration of the cluster, respectively.

For each cluster $c$, the value of $R_0$ ($R_{0c}$) was estimated and described using the median value of the estimates ($MR_{0c}$). Results were described using the median value of $R_{0c}$ because it is a measure of central tendency that is more robust, i.e. less sensitive to extreme values, than the mean. For that reason, $MR_{0c}$ was considered a robust estimator of the true value of the reproductive ratio ($R_0$) in the region. Note that $R_0$ refers to the parameter to be estimated, i.e. the 'true' disease reproductive ratio, whereas $MR_{0c}$ was the estimator of the parameter $R_0$, i.e. the result of a computation that was used to approximate the true, unknown value of the parameter $R_0$.

Evidence of spatial autocorrelation of $MR_{0c}$ was explored using Moran's $I$ test. The within- and between-cluster variation in the values of $R_{0c}$, were compared using an ANOVA test. A Grubbs' test was run to identify outliers in the series of $R_{0c}$ values computed. Non-significant differences ($P>0{\cdot}05$) in the results of Moran's $I$, ANOVA, and Grubbs' tests were interpreted as an indication that the values of $R_{0c}$ were homogeneous in the clusters.

## RESULTS

The value of $MR_{0c}$ ranged from 1·1 to 3·4 with a median estimate of 1·7 (Fig. 1). The largest value of $R_{0c}$ (5·8) was computed at the beginning of the cluster centred in Germany (Fig. 1, cluster 2). No significant differences in the value of $R_{0c}$ were estimated in the clusters (ANOVA test, $P=0{\cdot}12$), there was no evidence that the value of $MR_{0c}$ was spatially clustered (Moran's $I$ test, $P=0{\cdot}67$), and no outlier was detected in the series of $R_{0c}$ values (Grubbs' test, $P=0{\cdot}77$). Thus, the median value of $MR_{0c}=1{\cdot}7$ (1·1–3·4) estimated here was considered a robust estimate of the most likely value of $R_0$ over the course of the H5N1 HPAI epidemic in wild birds of Europe between 2005 and 2008. It should be noted that the value of $MR_{0c}=1{\cdot}7$ (1·1–3·4) was quantitatively similar to values of disease transmission estimated for the parameter in the wild bird population of Lake Constance in Switzerland ($R_0=1{\cdot}6$) [19], although the results of that study have not been published in the peer-reviewed international literature and for that reason, methods and assumptions of both approaches could not be compared.

The value of $MR_{0c}$ was similar for different geographical locations affected by the epidemic, as suggested by the results of the Moran's $I$, ANOVA, and Grubbs' tests. This finding could be explained, at least in part, by the observation that HPAI was transmitted through contamination of water in about 90% of the cases reported in aquatic species [20]. Therefore, it is possible that the values of $MR_{0c}$ were a consequence of general environmental and demographic conditions that are common to habitats populated by aquatic birds. Certainly, point estimates of $R_{0c}$ computed within each cluster were variable (Fig. 1) and probably influenced by a multiplicity of ecological, environmental, and epidemiological factors, such as variations in the population density, presence or absence of specific susceptible species, and changes in weather conditions. Such variation is consistent with the results of the ANOVA test, which suggest that variations in the value of $R_{0c}$ were greater within the clusters than between the clusters.

Interestingly, although estimates of the between-farm reproductive ratio ($R_f$) have been computed for HPAI epidemics in Italy in 1999–2000 ($R_f=1{\cdot}2-2{\cdot}7$) [21, 22], Canada in 2004 ($R_f=1{\cdot}4-3{\cdot}6$) [21], The Netherlands in 2003 ($R_f=0{\cdot}9-6{\cdot}5$) [21, 23]; and Romania ($R_f=1{\cdot}9-2{\cdot}7$) [13], to the best of our knowledge, the within-farm transmission rate of HPAI ($\beta$) has been estimated only using data from the epidemic that affected The Netherlands in 2003 ($\beta=4{\cdot}50$ per infectious chicken per day, 95% CI 2·68–7·57) [24]. Considering that the duration of infectiousness in poultry populations in The Netherlands in 2003 was estimated as 4 days [24], those results are equivalent to an average value of $R_0=18$, i.e. about tenfold greater than the value estimated here for wildlife. This difference is biologically sound because one would expect the value of $R_0$ to be greater in poultry farms than in wild bird populations as a consequence of the larger densities and contact rates observed in poultry compared with wild birds.

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## DISCUSSION

It should be noted that the methodological approach used here is probably unsuitable for the quantification of $R_0$ in poultry farms because of the effect that limited population size and high density would have on the transmission rate of the disease. However, the method may apply under certain conditions in which population data are not recorded in the national registry and for which it is possible to assume that population size is sufficiently large, such as the population of backyard or rural poultry of certain regions.

The justification to compute the value of $R_{0c}$ within each cluster is that cases that are closely located to each other in time and space are more likely to be associated to each other than remotely located cases. Thus, the value of $R_{0c}$ computed here could be interpreted as an approximation of local transmission of disease, because long-distance contacts were not considered. Note that because long-distance transmission was ignored in the computations, the procedure may have resulted in an underestimation of the value of $R_{0c}$. In turn, some of the cases computed within the cluster probably occurred as a consequence of migration of infected animals into the region, rather than to local disease spread, which would result in an overestimation of the values of $R_{0c}$. For those reasons, it is probable that both biases compensated each other and that the estimates of $MR_{0c}$ presented here approximated the true value of the parameter $R_0$.

A potential bias of the study is that $MR_{0c}$ was computed using the number of reported outbreaks, which, in most cases, corresponds to the number of dead birds in which H5N1 HPAI infection was confirmed. Thus, the criteria used for the case-definition here is highly specific (~100%, if it is assumed that no country will officially report a false-positive case of HPAI), but with uncertain, and probably low, sensitivity, because only a proportion of infected birds will be reported as cases. However, if the proportion of false-negative cases remains approximately constant throughout the periods of time investigated for each cluster, then the values of $MR_{0c}$ will remain relatively unbiased, even if the proportion of false-negative cases is high. It can be assumed that surveillance activities may have increased in a given area, after the detection of the initial cases, which could have resulted in the overestimation of the values of $R_{0c}$. Conversely, it is also possible that after the initial reporting of cases, not all the subsequent cases in a given region were reported, because the epidemiological situation of the region did not change, which would result in an underestimation of the value of $R_{0c}$. Thus, again, it is also possible that both biases compensated for each other, so that point estimates of $MR_{0c}$ remained relatively unbiased.

The approach used here to adjust for the influence of sources of bias and different epidemiological and ecological conditions, was to replicate the study in nine selected geographical areas and periods of time in which disease cases were clustered. Thus, one would expect that some biases and factors will result in the overestimation of $R_{0c}$ in some of the regions, and in its underestimation in others, so that the average value of the $MR_{0c}$ computed for each cluster will approximate the true value of the parameter $R_0$. Moreover, the values of $MR_{0c}$ were similar for all clusters, as indicated by the results of Moran's $I$, ANOVA, and Grubbs' tests, which was considered evidence of the robustness of the estimates of $MR_{0c}=1{\cdot}7$ ($1{\cdot}1-3{\cdot}4$) computed here.

Estimates of $R_0$ presented here could be useful to parameterize models for spread of HPAI in wild birds and to predict the impact of future epidemics. Ultimately, these results might help to design effective prevention and control programmes against HPAIV for the European poultry sector. The method presented here, which is an extension of techniques used to compute infectious disease transmission [17], could also be extended to estimate the value of $R_0$ for infectious disease epidemics in the absence of population data, which makes the approach particularly suitable for computation of the parameter in wildlife populations.

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## DECLARATION OF INTEREST

None.

## REFERENCES


1. **Alexander DJ.** A review of avian influenza in different bird species. *Veterinary Microbiology* 2000; **74**: 3–13.
2. **Terregino C, *et al.*** Active surveillance for avian influenza viruses in wild birds and backyard flocks in Northern Italy during 2004 to 2006. *Avian Pathology* 2007; **36**: 337–344.
3. **Munster VJ, *et al.*** Mallards and highly pathogenic avian influenza ancestral viruses, northern Europe. *Emerging Infectious Diseases* 2005; **11**: 1545–1551.
4. **De Jong MCM, *et al.*** Intra- and interspecies transmission of H7N7 highly pathogenic avian influenza virus during the avian influenza epidemic in the Netherlands in 2003. *Revue Scientifique et Technique* (*International Office of Epizootics*) 2009; **28**: 333–340.
5. **World Organisation for Animal Health (OIE).** World Animal Health Information Database Interface (WAHID). (http://www.oie.int/wahis/public.php?page=single_report&pop=1&reportid=8706). Accessed 20 December 2009.
6. **Artois M, *et al.*** Outbreaks of highly pathogenic avian influenza in Europe: the risks associated with wild birds. *Revue Scientifique et Technique* (*International Office of Epizootics*) 2009; **28**: 69–92.
7. **Keawcharoen J, *et al.*** Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). *Emerging Infectious Diseases* 2008; **14**: 600–607.
8. **Iglesias I, *et al.*** Environmental risk factors associated with H5N1 HPAI in Ramsar wetlands of Europe. *Avian Diseases*. Published online: 28 November 2009. doi:10.1637/8970-062609-Reg.1.
9. **Willeberg P, *et al.*** Visualization and analysis of the Danish 2006 highly pathogenic avian influenza virus H5N1 wild bird surveillance data by a prototype avian influenza bioportal. *Avian Diseases* 2010; **54**, s1: 433–439.
10. **Martinez M, *et al.*** Association between number of wild birds sampled for identification of H5N1 avian influenza virus and incidence of the disease in the European Union. *Transboundary and Emerging Diseases* 2008; **55**: 393–403.
11. **Dohoo I, Martin W, Stryhn H.** *Veterinary Epidemiologic Research*. Charlottetown, Prince Edward Island, Canada: VER Inc., 2010, pp. 722.
12. **De Jong MCM.** Mathematical modeling in veterinary epidemiology: why model building is important. *Preventive Veterinary Medicine* 1995; **25**: 183–193.
13. **Ward MP, *et al.*** Estimation of the basic reproductive number ($R_0$) for epidemic, highly pathogenic avian influenza H5N1 subtype spread. *Epidemiology and Infection* 2009; **137**: 219–226.
14. **World Organisation for Animal Health (OIE).** Terrestrial Animal Health Code, 17th edn, 2008 (http://www.oie.int/eng/normes/mcode/en_glossaire.htm#terme_unite_epidemiologique). Accessed 20 December 2009.
15. **Iglesias I, *et al.*** Identifying areas for infectious animal disease surveillance in the absence of population data: highly pathogenic avian influenza in wild bird populations of Europe. *Preventive Veterinary Medicine* (in press).
16. **Ward MP, Carpenter TE.** Analysis of time-space clustering in veterinary epidemiology. *Preventive Veterinary Medicine* 2000; **43**: 225–237.
17. **Anderson RM, May RM.** *Infectious Diseases of Humans: Dynamics and Control*. Oxford and New York: Oxford University Press, 1991, pp. 757.
18. **Minh PQ, *et al.*** Spatio-temporal epidemiology of highly pathogenic avian influenza outbreaks in the two deltas of Vietnam during 2003–2007. *Preventive Veterinary Medicine* 2009; **89**: 16–24.
19. **Saurina J.** Risk-based surveillance of avian influenza in Switzerland: wild birds and awareness (dissertation). (http://library.vetmed.fuberlin.de/ResourceList/details/172737). Accessed 10 December 2009.
20. **Roche B, *et al.*** Water-borne transmission drives avian influenza dynamics in wild birds: the case of the 2005–2006 epidemics in the Camargue area. *Infection, Genetics and Evolution* 2009; **9**: 800–805.
21. **Garske T, Clarke P, Ghani AC.** The transmissibility of highly pathogenic avian influenza in commercial poultry in industrialised countries. *PLoS ONE* 2007; **2**: e349.
22. **Manelli A, *et al.*** Transmission parameters of highly pathogenic avian influenza (H7N1) among industrial poultry farms in northern Italy in 1999–2000. *Preventive Veterinary Medicine* 2007; **81**: 318–322.
23. **Stegeman A, *et al.*** Avian Influenza A Virus (H7N7) epidemic in The Netherlands in 2003: Course of the epidemic and effectiveness of control measures. *Journal of Infectious Diseases* 2004; **190**: 2088–2095.
24. **Bos ME, *et al.*** Back-calculation method shows that within-flock transmission of highly pathogenic avian influenza (H7N7) virus in the Netherlands is not influenced by housing risk factors. *Preventive Veterinary Medicine* 2009; **88**: 278–285.