

Investigation of the Epidemiology of Avian Influenza in Wild Birds at Lake Constance – A Tri-lateral Project of Germany, Austria and Switzerland

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ABSTRACT

When highly pathogenic avian influenza (AI) H5N1 (HPAI H5N1) arrived in Europe in 2005, little was known about its ecology and epidemiology in wild birds. In this context, Switzerland initiated a research program to investigate avian influenza infections in wild birds at Lake Constance. The project was designed as a tri-national program involving scientists, government agencies and NGOs of the abutting countries of Lake Constance. This paper provides a descriptive analysis of AI in wild birds examined in the region of Lake Constance for a period of two years and compares the effectiveness of different surveillance methods in relation to order composition. The data was derived from dead bird recoveries in the course of passive surveillance as well as from live feral birds tested within active monitoring programs. Influenza A viruses of different subtypes but not HPAI H5N1 were found. Active surveillance proved to be more effective than passive surveillance for the detection of low pathogenic avian influenza virus (LPAIV) in water birds. Due to the small number of positive samples, the role of particular species as carriers could not be determined so that future surveillance programs should target all water birds. Positive samples were found between February 2007 and March 2008 showing significant seasonal variation. Nevertheless, further analyses are needed to understand the spatial and temporal dynamics of these LPAI cases.

KEYWORDS

Lake Constance, avian influenza, surveillance methods, spatio-temporal dynamics

INTRODUCTION

Avian influenza (AI) is an infectious disease caused by RNA viruses of the family *Orthomyxoviridae* and commonly affects birds including poultry. The virus is characterized by its genetic diversity, typed according to the combination of two surface proteins: the haemagglutinin (HA) and the neuraminidase (NA). The natural reservoir for these viruses is waterfowl, particularly dabbling ducks in which nearly all combinations of the 16 HA and 9 NA antigenic subtypes have been found (Latorre-Margalef et al., 2008). Some subtypes, particularly HPAI H5N1, pose a serious threat to animal health, wildlife conservation and human health as well as economic welfare. Various public authorities therefore supported the enhancement of the understanding of the behavior of influenza viruses in nature in order to develop risk-management policies and more efficient, risk-based surveillance designs. By the time HPAI H5N1 spread from East and Southeast Asia across central Asia into Europe and the area of Lake Constance in winter 2005/2006, Germany, Austria and Switzerland started a research program to investigate AI infections in wild birds, as very little was known about their dynamics, ecology and epidemiology. In this study, we provide some results of avian influenza in wild birds examined in the region of Lake Constance for a two-year period. We also address the question whether some species play a role as carriers. Comparing active and passive surveillance methods, we show that active surveillance is more effective detecting LPAI infections. Based on the temporal distribution of virus detection, we hypothesized that there was a temporal clustering of influenza A infections.

MATERIAL AND METHODS

Study site and data collected

Lake Constance is the third largest freshwater lake in Central Europe and is located on the borders of Austria, Germany, and Switzerland. The study site included the lake and surrounding areas within a 30 km radius. Lake Constance provides an important wetland habitat for wild birds. A population of 20,000 water birds of many different species is resident year-round. For migratory birds, the lake provides an important wintering site. The number of water birds may exceed 250,000 during winter (Heine et al., 1999). The extent of migration and the degree of mixing during migration and wintering are highly variable among species. Within certain species (e.g., Mallard [*Anas platyrhynchos*]) some subpopulations are resident, while others are migratory. Swab samples taken from birds that were either caught by a swim-in-trap run by the research program, shot in the course of hunting, dead recoveries, or mute swans caught during molting season provided the basis of the data analyzed. Samples were sent to regional or reference laboratories where a

TaqMan one-step real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) method was used to amplify part of the highly conserved matrix protein gene typical of the influenza A viruses (Spackman 2002). If positive for influenza A, specific rRT-PCR was used to detect the HA gene of subtype H5, H7 and N1. At last, the isolated virus was sequenced (Zou 1997).

Data management and analysis

The test result as well as other relevant information about each tested bird was then entered in a central database. Descriptive statistical analysis was performed with Microsoft Excel. The Geographical Information System (GIS) package ArcView 9.2 (Environmental Systems Research Institute, Redlands, CA) was used to create maps documenting the spatial distribution of tested birds and AIV positive samples in each municipality. Crude estimates of apparent prevalence of AIV among tested birds were calculated for each municipality. In order to identify surveillance methods that are effective in detecting avian influenza virus of any subtypes, the condition of the tested birds were grouped into „active surveillance“ – containing samples of birds tested alive or shot in the course of hunting – and „dead recovery“ – covering all birds tested dead and sick, respectively. Disregarding possible covariates, a Monte Carlo permutation test was applied. In order to consider possible covariates, a stratified analysis in the form of a Cochran Mantel Haenzel Test was run to strengthen the first statement concerning the effectiveness of certain surveillance methods.

RESULTS

The sampling of feral birds began at the beginning of September 2006 and continued until the end of August 2008. Samples were collected from 2,003 feral birds that were either found dead or sick, respectively, hunted or caught in the region of Lake Constance. 46 samples tested positive for Influenza A of different subtypes but not HPAI H5N1. No valid test results were available for 58 samples. The total prevalence of LPAI virus in all waterfowl sampled during the two-year period was 2.4%.

LPAI virus was isolated in four species: Mute Swan (*Cygnus olor*), Mallard (*Anas platyrhynchos*), Common Coot (*Fulica atra*) as well as Common Teal (*Anas crecca*) (20%). Positive samples were also obtained from birds identified only as "wild duck". The prevalence of LPAIV among tested birds of each of the species is shown in Table 1.

Table 1 The species-specific prevalence of LPAIV in wild birds sampled in the region of Lake Constance between September 2006 and August 2008.

Latin name	Common Name	Positive	Total Sample	Population ¹
<i>Cygnus olor</i>	Mute Swan	25 (3.6 %)	687	1,277
<i>Anas platyrhynchos</i>	Mallard	10 (1.9 %)	510	15,443
<i>Fulica atra</i>	Common Coot	3 (1.7 %)	176	41,055
<i>Anas crecca</i>	Common Teal	7 (20 %)	35	2,855
-	"wild duck"	1 (4.3 %)	23	-

¹ Mean of January counts of the birds of Lake Constance 1982-1997 (Heine et al., 1999)

The first LPAIV positive case was a mute swan hunted on the 19th February 2007 and the last LPAIV positive bird was found dead on 10th March 2008. The highest prevalence of Influenza A was found between August and November 2007. Wild birds were sampled from 99 municipalities in the region of Lake Constance. The number of birds sampled varied greatly between municipalities, ranging from one to 351. Positive samples were obtained in 11 municipalities. The crude local prevalence of LPAI ranged from 0 out of 131 samples to 10 out of 38 (26.3%).

About one sixth of all samples were collected within passive surveillance programs; however, all but two positive samples originated from birds tested within active monitoring i.e. caught birds tested alive or hunted birds tested dead (2.7%). In contrast, with only 0.6% of positive samples from dead recoveries, the passive surveillance had a significantly lower prevalence (Permutation test, $\chi^2 = 5.3$, $p = 0.02$). After adjustment for order composition, active surveillance tended to be more effective than passive surveillance but the evidence was weak ($\chi^2 = 2.4$, $p = 0.11$).

DISCUSSION

The total prevalence of LPAI virus in all waterfowl sampled during the 2-year period was 2.4%. This overall prevalence was similar to that found in surveillance programs of other European countries (SANCO, 2008). The prevalence in all mallard samples was approximately 2%. Although this species is known to play a central role in the perpetuation of AIV in nature, mallards in the area of Lake Constance seem to play a minor role in the context of AIV infections (Wallensten et al. 2007). Three swab samples of coots caught in the same municipality on the same day returned positive for LPAI virus. No other positive samples were obtained at that point of time. A local incidence and a limited transmission of AI virus are therefore assumed.

Based on this low prevalence in which is in accordance to that found in other studies (SANCO, 2008), coots can be considered innocuous in perpetuating avian influenza. The prevalence of LPAI virus in mute swans ranged from zero to approximately 8% in August 2007. Both facts, that such a high prevalence has not been detected in other studies and the comparison of this prevalence to those of other months indicate that LPAI virus was introduced into the local swan population. All of the samples of teals were collected during hunting season in fall 2007. The prevalence therefore represents the status of influenza infection in this species at that time of year. But as the sample size is rather low and teals were sampled neither before nor after this particular hunting season, no conclusion on the potential role of common teals as carrier can be made.

The analysis of the temporal distribution showed that some months have a significantly increased disease rate for AIV. This could be due to seasonality, risk factors that haven't been identified so far or even sampling bias. The spatial analysis has just started. The crude prevalence may be misleading as the variance associated with the estimated prevalence is influenced by sample size: small sample sizes are associated with greater variance and uncertainty. Further analyses are needed to overcome the variability in prevalence associated with small sample sizes.

In accordance to other studies, we found that active monitoring for AI is an effective method for detecting LPAI virus infections (Globig et al., 2009). We see the strength of this method in the potential of actively defining the species to target and in the potential of determining the time of taking samples (for example during bird migration).

CONCLUSION

Based on the small number of positive samples no particular species was identified to act as a carrier of AIV. Future surveillance programs should therefore target all waterbirds. Although there is evidence of virus introduction, further analyses are needed in order to understand the spatio-temporal dynamics of LPAI cases found in the region of Lake Constance. Active surveillance is a good method for detecting LPAI infections but it is found to be inferior to passive surveillance when aiming at the detection of HPAIV H5N1 in wild birds.

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