Confidence within limits- Introduction of highly mobile pathogens into Germany and their early detection.

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The detection of highly pathogenic Influenza-A- Virus HPAI H5N1/Asia in wild migratory birds at the coast of the Baltic Sea in February 2006 and successive outbreaks in wild birds and poultry holdings in 2006 emerged as a booster for a nationwide monitoring program in wild birds. Initial goals have been (i) the description of bird species affected and involved in transmission, (ii) the description of basic epidemiological key figures and (iii) the identification of an endemic status and the detection of new outbreaks. The general outline of the resulting dataset includes the taxonomic identification of 514 bird species or groups of birds in a specially created hierarchical key and the geographical designation on municipality level. The sampling of sick or dead animals as well as living or hunted birds allows for both a passive and an active part of the monitoring. When cases of avian influenza in wild birds are detected, the assessment of the true number of affected, positive animals is often difficult if not impossible. This results in limitations in calculating prevalence estimates. In addition, surveillance data in wild animals imply problems of autocorrelation in time and space, which affects the calculation of confidence limits. When the large number of different bird species is taken into account, it becomes obvious that a profound analysis of these data is a challenge for biometricians and veterinary epidemiologists. For the evaluation of the surveillance system, we propose a model for estimating confidence intervals for (i) prevalence calculations in outbreak situations (Baltic Sea and Wachenroth) or (ii) the absence of disease in certain time intervals for a specified regional unit in Germany (Lake Wusterhausen).

Elements of our model for confidence estimation:

- Definition of a geographical feature to be analyzed.
- Definition of a buffer –end of reporting monitoring results for that feature and that time period.
- Time series analyses with upper 95% confidence limit.
- Linear adjustment for the spatial distance from the centroid of the municipality to the feature.
- Adjusted for the species sampled by two scores each for active and passive sampling.
- Definition of a time effect of the sampling and moving averages for the sampling frequency.

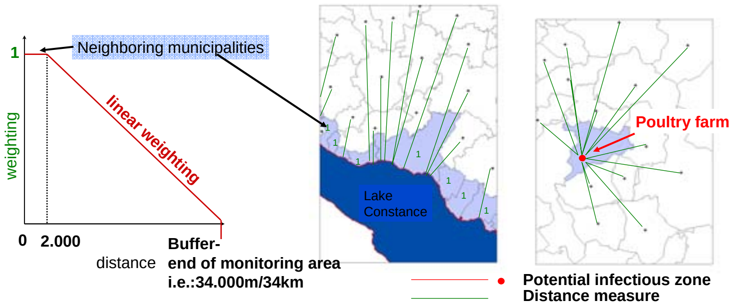

Fig.1-3: Geographical features and linear weighting for the distance of the samples.

Two indices for weighting the 514 wildbird-species in Germany:

Scoring: **1**(very low); **2** (low); **3**(neutral, medium); **4**(high); **5**(very high, optimal)

Morbidity/Mortality-Index (**MM**):
Measurement for the ability of the species to show clinical signs in reaction to H5N1. Susceptibility
Designation: passive monitoring (dead or sick birds)
Aim: detection of virus/prevalence
Typical: mute swan, black-necked grebe, canada goose =5

Transmission-Index (**TM**):
Measurement for the ability of the species to transmit and distribute H5N1. Being infectable without clinical signs and replicate the virus. Migratory status (long- medium- short range)
Designation: active monitoring (living or hunted birds)
Aim: detection of virus transmission
Typical: mallard =5

There is increasing knowledge on the susceptibility of some species for H5N1 and extensive data on the movement of migratory birds provided by ornithologists. By contrast, information on the potential role of most of the bird species in the transmission of H5N1 is scarce. Scores were introduced that were preliminary estimated from known data, literature, animal testing and expert opinion for this model. For most species in our dataset no information is available (score=3). In the future more data and knowledge has to be incorporated to justify the scores. The scoring system is used in an ordinal fashion in the model but should be interpreted carefully as an indicator since one high score (5) is "worth" 1.67 neutral scores (3). Insufficient data for 514 different species regarding most of the criteria required to define an informative score do not allow a finer scoring at this stage.

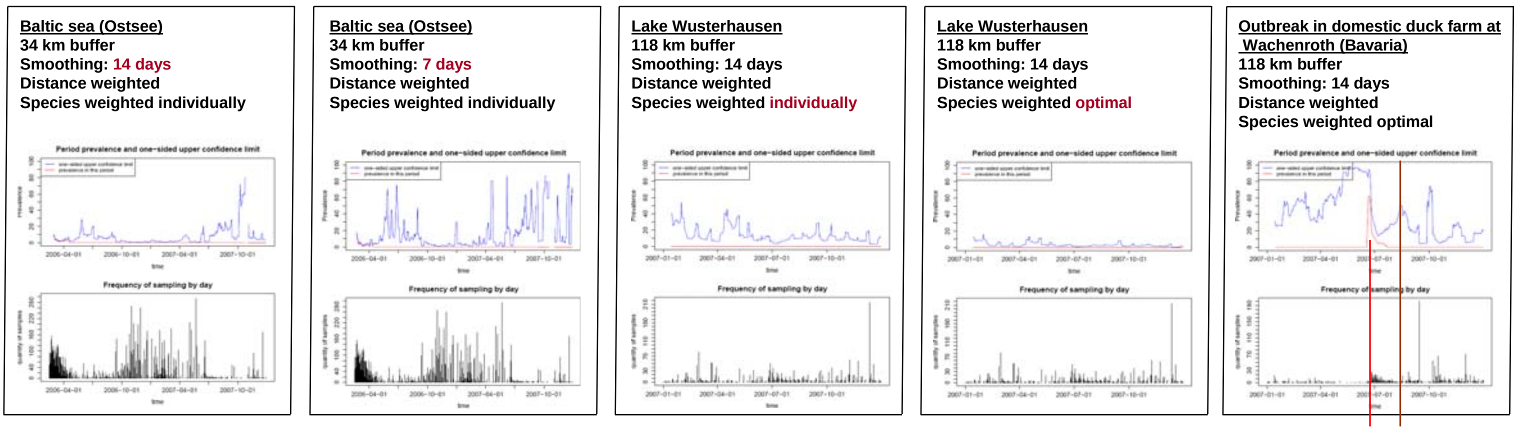

Fig.4-8: Upper: period prevalence and one-sided 95% confidence limit for different sites. Bottom: Daily frequency of tests for different regions. The values for the smoothing window and the species weighting are different to allow comparisons. Fig.8: Outbreak in swans at Lake Wöhrder (red), outbreak at domestic duck farm (brown).

Results:

The dataset of the wild bird surveillance in Germany for the years 2006 to 2007 contains 61,126 entries. 268 out of 514 possible species and groups of species were tested. Most of the samples were collected from wild ducks, wild goose, raptors/bird of prey and swans. Passive surveillance accounts for 58.8% of the samples and the active part for 41.2%. Most positive samples (H5N1) were collected from dead or sick birds. Only one case was found in a hunted swan during an outbreak situation at Lake Kelbra. Allocation of the monitoring activities to the federal states leads to a general distribution over Germany, but irregular sampling at the level of the counties produces a dataset with spatial autocorrelation (spatial data not shown). In addition, infrequent sampling produced datasets with high autocorrelation in time (Fig. 4-8). Outbreak situations in wild birds (i.e. at the Baltic Sea during winter 2006) led to prevalence estimates that must be carefully interpreted against the background of sampling and reporting bias as well as missing data.
For most places and time intervals, the upper 95% confidence limit did not fall below 10% (Fig.: 4-8). Only intensive monitoring between November 2006 and June 2007 at the Baltic sea led to reliable confidence limits over a long period (Fig.: 4). Between the outbreaks of wild swans at Lake Wöhrder and in domestic ducks in Wachenroth, the upper confidence limit varied considerably (appr. 25%). Different smoothing windows created different model interpretations with a varying degree and stability of confidence (Fig.:4+5). When the species scores were taken into account, the surveillance system could be evaluated in terms of the effectiveness of the birds sampled under the respective conditions, but many samplings can only be considered as "very low" (=1) or "low" (=2) in any of the two scores (Tab.1). Optimizing the sampling can lead to higher confidence in surveillance system regarding virus prevalence and virus transmission activity (Fig. 6, 7).

	1	2	3	4	5
Baltic Sea	2410	1864	3921	1898	1857
Lake Wusterhausen	372	550	759	186	379
Wachenroth	289	19	246	142	827

Tab.1:Distribution of weighting scores in different sampling regions

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Conclusions:
Limited resources did not allow installing a reliable surveillance system for Germany as a whole and the variety of species over a long period. In the future, it may be possible to develop a risk-based approach and to target specific animals with a better chance of detecting the virus. To this end, more information for bird movement and susceptibility to H5N1 is needed to improve the scoring of the different wild bird species.
An open questions remains, how much confidence is created by a single sample. This is important as the information is needed for a better and scientifically profound definition of the smoothing window.
The model described in this paper may prove useful for the detection of time intervals, geographical units and population subgroups where monitoring is insufficient to detect a pathogen present at a low prevalence. Altogether it may be suitable as a tool for assessing the risk of introduction of Influenza-A viruses into poultry and the food-chain in Germany.

Formula notation:

Distance weighting:

$we = 1$ =in neighboring (shore) municipalities and <2000 m distance

$$we = \frac{(0.01-1)\times(D-2000)}{(B-2000)}$$ =other municipalities

D= distance in Meter
B= buffer in Meter (Surveillance zone)

Species weighting:

$$ws = \frac{S}{3}$$

S= Point in Score
3 point in the score are a "Standard sample"; 5 points are 1.67 Standard samples