Towards a new, ecologically targeted approach to monitoring wild bird populations for avian influenza viruses

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SUMMARY

Prevalence monitoring of avian influenza in wild bird populations is important to estimate risks for the occurrence of potentially zoonotic and economically disastrous outbreaks of highly pathogenic avian influenza virus (AIV) in poultry worldwide. A targeted, cost-effective monitoring method for AIV in wild birds was developed, which is based on monitoring results for AIV in Germany and information on the distribution and abundance of wild bird species in selected habitat types. Spatial data were combined with virological and outbreak data for the period of 1 January 2006 to 31 December 2010. Using Germany as an example, we identified 11 indicator species. By concentrating monitoring efforts on these species in spatially confined locations, we propose a targeted and more cost-effective risk-based AIV monitoring approach that can be adapted universally for the identification of wild bird indicator species worldwide with the perspective of reducing sample sizes (and costs) without impairing the validity of the results.

Key words: Avian influenza, indicator species, Ramsar sites, risk-based sampling, wild bird database, wild bird monitoring.

INTRODUCTION

Wild birds constitute the natural reservoir of avian influenza viruses (AIV) of low pathogenicity (LPAIV) [1–3]. This huge AIV gene pool has the capability of producing viruses with zoonotic and even pandemic potential [2, 4]. In addition, the LPAIV of subtypes H5 and H7 might act as precursor viruses for highly pathogenic avian influenza viruses (HPAIV). These may arise through mutation following transmission and circulation of LPAIV in gallinaceous poultry and can be re-transferred from HPAIV-infected poultry to wild birds [4]. Therefore, wild bird monitoring for AIV has been compulsory throughout the European Union (EU) from 2005 until 2010 [5, 6]. To manage and analyse the

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information obtained through the German wild bird monitoring programme for AIV, a database (AI-DB) was established at the Institute of Epidemiology of the Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, in Wusterhausen, Germany [7, 8]. Each sample from a wild bird tested was registered, including species, occasion and location of sampling as well as data on laboratory results that were uploaded onto the database at regular intervals by the respective veterinary authorities of the 16 German Federal States. Any sample positive for AIV by M gene-specific real-time reverse transcriptase polymerase chain reaction (M-RT-qPCR) [9] was characterized centrally at the National Reference Laboratory for Avian Influenza to the haemagglutinin (H1–H16) and neuraminidase (N1–N9) level and, in case of subtypes H5 or H7, also with regard to pathotype [7]. Samples for AIV identification were provided through active and passive wild bird monitoring. As part of the active monitoring, samples originating from legal hunting of wild bird species constitute an important contribution to the positive identification of H subtypes [10, 11]. Within 24 h after identification of a (suspected) case or outbreak of notifiable avian influenza in wild birds or poultry in Germany a notification has to be uploaded onto the national animal disease notification system database (Tierseuchennachrichtensystem; TSN) by the competent veterinary authority at district level [12, 13]. However, blanket monitoring systems, which have been co-financed by the EU since 2005, have proved to be costly, e.g. €857 164 in the study period (1 January 2006 to 31 December 2010). In view of the low sensitivity of active monitoring, in contrast to passive monitoring, for the detection of HPAIV [7, 8] co-financing for active monitoring has been cut. Yet, active monitoring was highly successful in identifying LPAIV including H5 and H7 subtypes which is vital for a continuing pro-active supervision of developments in the AIV gene pool. The objective of this study was to identify wild bird indicator species as a basis for a more targeted and economical monitoring approach for AIV infections in spatially confined wetland habitats (Ramsar sites) in Germany [10, 13, 14].

MATERIALS AND METHODS

Active and passive wild bird monitoring

Wild bird monitoring for the detection of LPAIV and HPAIV in Germany was performed according to the definition as given in the EU DG-SANCO 'Guidelines on the implementation of survey programmes for avian influenza viruses in poultry and wild birds to be carried out in Member States in 2007' [5]. This document asks specifically for an increase in (a) active surveillance in living or hunted birds, in particular species resident in or migrating from areas affected by HPAI H5N1 avian influenza outbreaks and identified as presenting a higher risk for avian influenza transmission, and (b) passive surveillance on wild birds found dead [5, 6]. The document further states that 'the design of the survey will need to be adapted to the national situation as regards selection of species to be sampled according to species predominance and bird population sizes' [5]. In Germany, therefore, it is the responsibility of each of the 16 Federal States to implement these guidelines accordingly.

Species identification and ranking

Geographical and wild bird species information on all 34 Ramsar sites in Germany was obtained from the official Ramsar website [14]. Ramsar sites are wetlands of international importance. Their protection is based on the 'Ramsar Convention', an international treaty adopted in 1971 in the Iranian city of Ramsar that came into force in 1975 [15]. Its mission is 'the conservation and wise use of all wetlands through local and national actions and international cooperation, as a contribution towards achieving sustainable development throughout the world' [15]. We selected these Ramsar sites as our ecologically targeted zones 'due to the well-known reservoir function of wild birds (in particular of migratory waterbirds) for all LPAI subtypes' and 'in order to detect LPAI H5 and H7 subtypes which may pose a risk to poultry' [5]. All administrative districts in Germany at the 'nomenclature for territorial statistics (NUTS)' 3 level [16], which encompass at least part of a Ramsar site (Ramsar districts), were identified, and the five most abundant wild bird species for each Ramsar site determined. Further bird species that might have been present, but for which information on their abundance was not available, were not considered [14, 15]. Each of these wild bird species was then ranked with regards to occurrence in overall number of German Ramsar sites [14, 15]. For example, in 13 out of 34 different German Ramsar sites mallards (*Anas platyrhynchos*) are among the five most prominent species (Fig. 1).
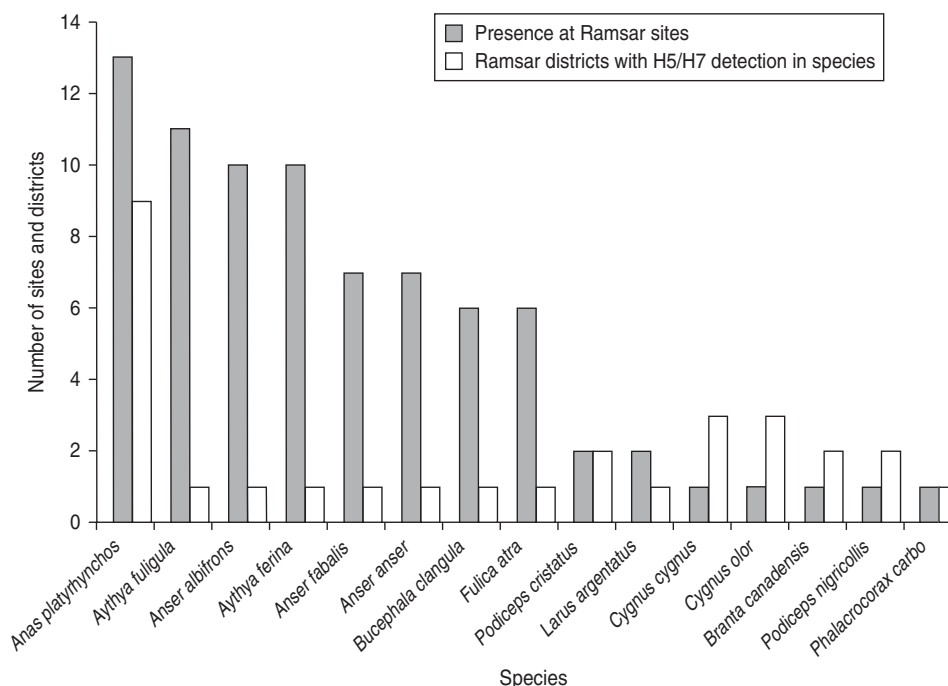
Fig. 1. Selected wild bird species present at ⩾1 Ramsar site with positive H5/H7 identification in Ramsar districts. The selected wild bird species belong to the five most abundant species at ⩾1 Ramsar sites and tested positive for H5/H7 in Ramsar districts from 1 January 2006 to 31 December 2010, in Germany. *Anas platyrhynchos* (Mallard Duck), *Aythya fuligula* (Tufted Duck), *Anser albifrons* (White-fronted Goose), *Aythya ferina* (Common Pochard), *Anser fabalis* (Bean Goose), *Anser anser* (Greylag Goose), *Bucephala clangula* (Common Goldeneye), *Fulica atra* (Eurasian Coot), *Podiceps cristatus* (Great Crested Grebe), *Larus argentatus* (European Herring Gull), *Cygnus Cygnus* (Whooper Swan), *Cygnus olor* (Mute Swan), *Branta canadensis* (Canada Goose), *Podiceps nigricollis* (Black-necked Grebe), *Phalacrocorax carbo* (Great Cormorant).

Collation of virological data

Using data from AI-DB and TSN, we identified species that had tested positive for H5/H7, regardless of the pathotype, in the whole of Germany, not only at selected Ramsar sites, during the study period of 1 January 2006 to 31 December 2010 [10, 13]. We also included in this approach all findings from the passive wild bird monitoring efforts throughout the country. Furthermore, information from the AI-DB gave an overview on all AIV haemagglutinin subtypes (H1–H16) identified in wild bird species in Germany during the study period [10]. In 2006, AIV test results for sampled wild birds could be submitted to the AI-DB on a voluntary basis. As a consequence, not all Federal States submitted their respective findings for this year. Information on positive findings of HPAIV H5 or H7 in wild birds missing in the AI-DB for 2006 was therefore retrieved from TSN. Since 2007, the submission of wild bird data on AIV findings to the AI-DB was compulsory [6]. Finally, through analysis of data from AI-DB and TSN we determined all administrative districts (NUTS 3) where H5/H7 viruses had been identified in wild bird or domestic poultry

species in Germany during the study period (Fig. 2) [16]. These numbered 80 of a total of 402 districts in Germany, or about 20% [10, 13].

Data integration

Indicator species were identified by combining the above described sets of information and applying the following three selection criteria: the wild bird species (1) belongs to one of the five most abundant wild bird species at one or more Ramsar sites, and (2) shows at least one detection of AIV subtypes H5 or H7 at the respective Ramsar districts in combination with (3) a level of detection of any H subtypes present in this species ⩾0·5%. The legal hunting periods for these indicator species according to Federal State law were also determined [17].

RESULTS

Ramsar districts and identification of H5/H7

Most of the German Ramsar sites (*n* = 34) are located in Bavaria (*n* = 8), whereas none is located in the
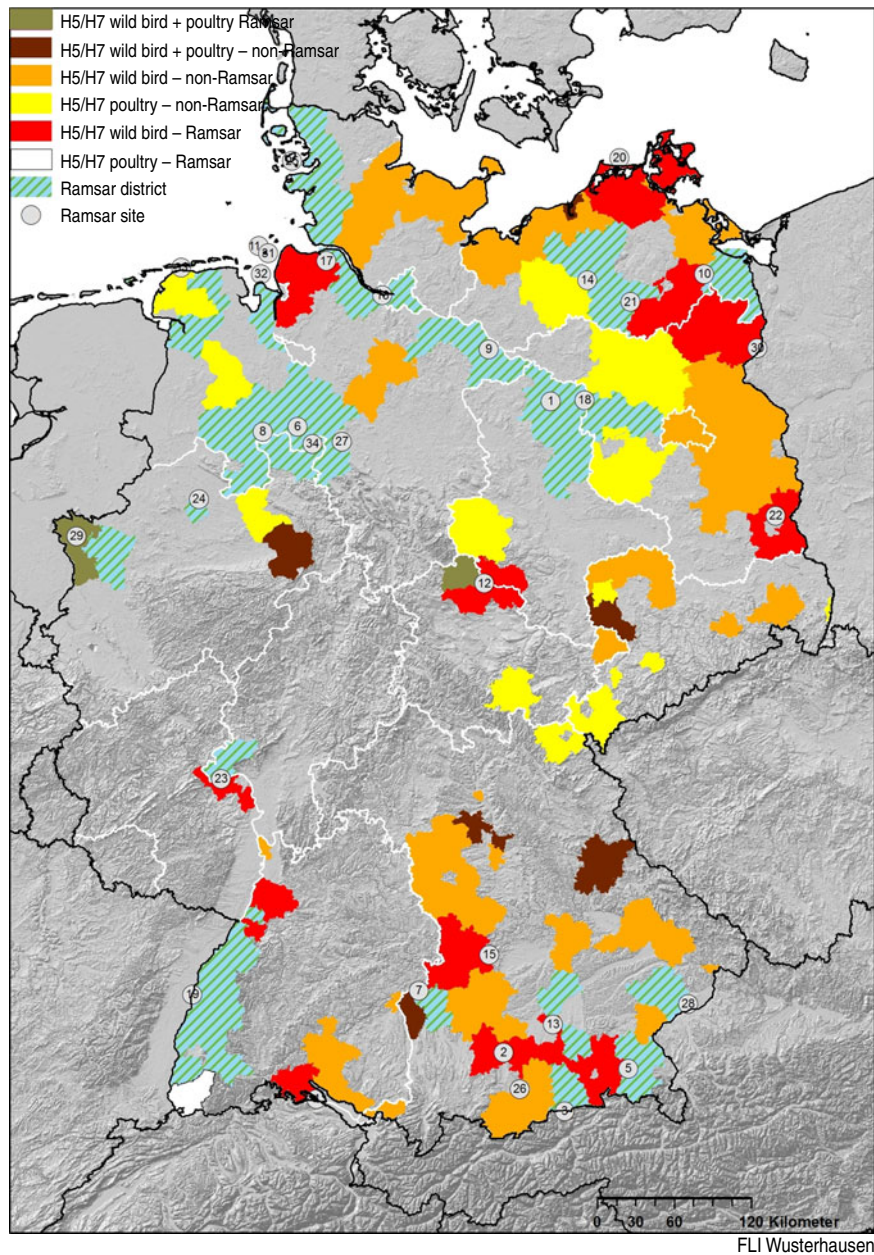
Fig. 2 [*colour online*]. Administrative districts where H5/H7 was detected in wild birds or poultry from 1 January 2006 to 31 December 2010, compared to the distribution of Ramsar districts in Germany. The numbers 1–34 (within a circle) refer to the 34 Ramsar sites in Germany.

Federal States of Berlin, Bremen, Saarland and Saxony. Some Ramsar sites are shared between different districts belonging to two or more Federal States (Fig. 2). Overall, a total area of 838 226 ha (2·43%) is protected under the Ramsar convention in Germany [14]. Figure 2 shows all administrative districts encompassing at least part of a Ramsar site (referred to as 'Ramsar district'). In addition, districts where AIV H5/H7 has been identified in wild bird or poultry species in Germany during the study period

are depicted. While outbreak districts could be geographically close to each other, cases in wild birds and outbreaks in domestic poultry occurred at different times and were caused by different H5 subtypes in these neighbouring districts (for examples see Fig. 2).

Indicator species

Altogether 25 species were identified with a positive HPAIV H5N1 result by active and passive wild bird
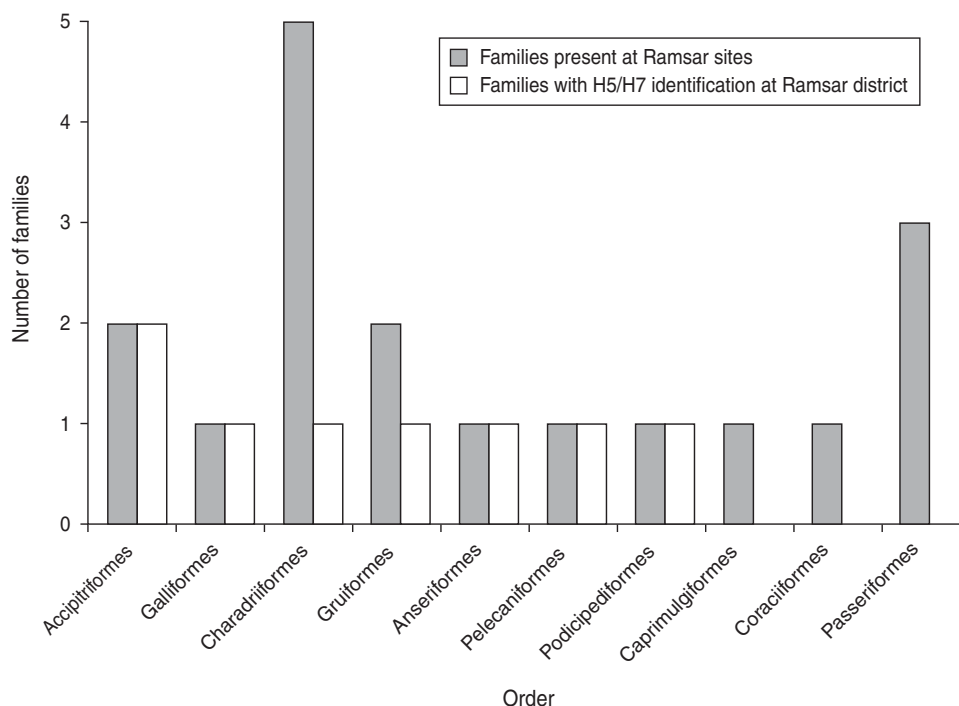
Fig. 3. Number of bird families with identification of H5/H7 in Ramsar districts compared to bird orders present at Ramsar sites. The number of wild bird and poultry families belonging to the bird orders of the five most abundant wild bird species at Ramsar sites were compared to the number of families per order with positive H5/H7 identification in Ramsar districts from 1 January 2006 to 31 December 2010, in Germany. Accipitriformes (birds of prey), Galliformes (gallinaceous birds), Charadriiformes (shorebirds and relatives), Gruiformes ('crane-like' birds), Anseriformes (ducks, geese, swans and relatives), Pelecaniformes (pelicans, tropicbirds, cormorants and relatives), Podicipediformes (grebes), Caprimulgiformes (nightbirds), Coraciiformes (kingfishers and relatives), Passeriformes (perching birds).

monitoring throughout the whole of Germany during the study period [10, 13]. Of those we identified there were 15 wild bird species, each of which belonged to one of the five most abundant wild bird species at one or more Ramsar sites, and showed at least one AIV H5/H7 detection event at the respective Ramsar district. Mallards (*Anas platyrhynchos*) are the most prominent species as far as abundance at Ramsar sites ($n=13$) and H5/H7 prevalence in Ramsar districts ($n=9$) are concerned. Eight species were present at six Ramsar sites or more with, apart from mallards, detection of AIV H5/H7 in one Ramsar district. Among the species present only at one or two sites each (Fig. 1), Great Crested Grebes (*Podiceps cristatus*), Mute Swans (*Cygnus olor*), Whooper Swans (*C. cygnus*), Canada Geese (*Branta canadensis*), and Black-necked Grebes (*P. nigiricollis*) had tested positive for AIV H5/H7 in more than one Ramsar district. When investigating all Ramsar districts for the presence of AIV H5/H7 in any bird species (wild and domestic) present in the respective district, representatives of seven different orders were identified (Fig. 3). Two of those, namely Accipitriformes, and Galliformes, do

not fall under our definition of an indicator species. European Buzzard (*Buteo buteo*), Peregrine Falcon (*Falco peregrinus*) and Eagle Owl (*Bubo bubo*) are the only raptor species so far identified with a positive result for AIV H5/H7, while turkeys (*Meleagris gallopavo*) are the only domestic species with such a positive result in a Ramsar district in Germany [13–15]. The orders Passeriformes (perching birds), Caprimulgiformes (nightbirds), and Coraciiformes (kingfishers and relatives), although encompassing species which belong to the five most abundant wild bird species at Ramsar sites, did not test positive for AIV H5/H7. Furthermore, the two latter orders are markedly underrepresented in wild bird monitoring in Germany during the study period [10]. Finally, among the 15 wild bird species, nine indicator species were identified that had tested positive for any H subtype in at least 0·5% of the cases (Table 1).

Hunting

During the study period samples from hunted wild birds have contributed the highest percentage of

Table 1. *H subtypes detected in wild bird species during active and passive monitoring in the whole of Germany from 1 January 2006 to 31 December 2010*

Species	IS*	Samples	H+†	%	95% CI§	H1–H16	No.¶	H5/H7‖
Wild bird species with all year closed hunting season in Germany								
Black-necked Grebe (*Podiceps nigricollis*)	x	305	257	**84·3‡**	79·8–88·0	H4, H5	3	**H5N1**, H5Nx
Great Crested Grebe (*P. cristatus*)	x	301	40	**13·3**	9·8–17·6	H5, H11	2	**H5N1**
Whooper Swan (*Cygnus cygnus*)	x	897	29	**3·2**	2·2–4·6	H5, H6	2	**H5N1**, H5Nx
Common Goldeneye (*Bucephala clangula*)	x	33	1	**3·0**	0·1–16·0	H5	1	**H5N1**
Goosander (*Mergus merganser*)		93	0					
Wild bird species with open hunting season in Germany								
Tufted Duck (*Aythya fuligula*)	x	543	12	**2·2**	1·2–3·8	H5	12	**H5N1**, H5Nx
Mute Swan (*C. olor*)	x	4554	94	**2·1**	1·7–2·5	H4, H5, H7, H11	4	**H5N1**, H5N2, H7Nx
Canada Goose (*Branta canadensis*)	x	1645	15	**0·9**	0·5–1·5	H5, H11	2	**H5N1**
Mallard Duck (*Anas platyrhynchos*)	x	14 710	99	**0·7**	0·6–0·8	H1–H11	25	**H5N1**, H5N2, H5N3, H7Nx
White-fronted Goose (*Anser albifrons*)	x	1769	9	**0·5**	0·3–1·0	H1, H5, H6	5	H1N1, H5N2, H5Nx
European Herring Gull (*Larus argentatus*)		959	2	0·2	0·0–0·8	H5	1	**H5N1**
Greylag Goose (*A. anser*)		7163	9	0·1	0·1–0·2	H2, H5, H6, H9	5	**H5N1**
Eurasian Coot (*Fulica atra*)		3573	4	0·1	0·0–0·3	H5	2	**H5N1**, H5Nx
Bean Goose (*A. fabalis*)		1235	1	0·1	0·0–0·5	H5	1	**H5N1**
Wild bird species without hunting season in Germany								
Raptors (Falconiformes)		942	4	0·4	0·1–1·1	H5	1	**H5N1**
Peregrine Falcon (*Falco peregrinus*)	x	92	2	**2·2**	0·3–7·0	H5	1	**H5N1**
Eurasian Buzzard (*Buteo buteo*)	x	2444	11	**0·5**	0·2–0·8	H5	1	**H5N1**

x, Additional species for focused wild bird monitoring in Germany.

* Wild bird indicator species.

† Detection of H subtype.

‡ This bird species showed positive results for all H subtypes of ≥0·5%.

§ 95% confidence interval for H subtype prevalence [25].

¶ Number of H subtypes detected, bold face (H5/H7) indicates HP pathotype.

‖ H5/H7 subtypes detected in wild bird species during monitoring in Germany from 1 January 2006 to 31 December 2010.

Table 2. *Odds and risk ratios for the results of active and passive wild bird monitoring compared to the results of active monitoring in hunted wild birds only in Germany from 1 January 2006 to 31 December 2010*

	AIV positive	AIV negative	Total
Hunting	418	8810	9228
Non-hunting	2216	111 008	113 224
Total	2634	119 818	122 452
Odds ratio	2·4		
Risk ratio	2·3		
	H5/H7 positive	H5/H7 negative	Total
Hunting	69	9159	9228
Non-hunting	1037	112 187	113 224
Total	1106	121 346	122 452
Odds ratio	1·2		
Risk ratio	1·0		

AIV-positive results to the AI-DB (4·53% of the total amount of hunting samples and 15·87% of all AIV-positive samples), while at the same time providing only 7·54% of all samples (Table 2) [17, 18]. During the study period 0·85% of samples (from active and passive wild bird monitoring) showed a positive identification of H5/H7 subtypes, while in actively hunted wild birds this number was 0·75%. The odds [27] of showing an AIV-positive result were 2·4 times higher in actively hunted wild bird species than in wild birds sampled in the total active and passive blanket monitoring approach in Germany (Table 2). However, the detection of H5/H7 subtypes did not show any difference in the odds and risk ratios between these two groups (Table 1) [10, 13, 17, 18].

Haemagglutinin subtypes

When evaluating the distribution of influenza A virus haemagglutinin subtypes in the wild bird population throughout Germany, the following picture emerged: the H5 subtype was identified in 12 Federal States, while the H7 subtype was only detected in six of the 16 Federal States (Table 3). Of the remaining 12 subtypes identified in Germany so far, six were found in more than five Federal States (H1, H3, H4, H6, H9, H11); with H3 being the most widespread in nine Federal States (Table 3). The AI-DB data showed no detection of haemagglutinin subtypes H14 and H15 in wild bird species in Germany during the study period (Table 3). The broadest variety of haemagglutinin subtypes was detected in Mecklenburg-Western Pomerania ($n=12$), followed by Baden-Wuerttemberg ($n=11$), Bavaria ($n=11$), Brandenburg ($n=8$), North Rhine-Westphalia ($n=8$), and Rhineland-Palatinate ($n=8$) (Table 3). Table 3 also gives an overview on the distribution of the haemagglutinin subtypes according to wild bird groups: wild (dabbling and diving) ducks were the group with the largest variability of H subtypes detected so far ($n=12$; H1–H12), followed by swans ($n=8$; H1, H2, H4–H7, H11, H13), and wild geese ($n=8$; H1, H2, H5–H7, H9, H11, H13). H5 constituted the H subtype that was present in the largest number of wild bird groups ($n=7$), namely wild ducks, swans, wild geese, grebes, waders, raptors, and other species (Table 3).

DISCUSSION

The importance of wild birds as a reservoir of the influenza A virus gene pool has repeatedly been emphasized [1–3, 5, 19, 20]. As shown in Figure 2, in Germany Ramsar sites as selected wetland areas have played an important role as reservoirs for the detection of H5/H7 subtypes in wild birds as part of active and passive wild bird monitoring. Since 2006, intensive and costly nationwide wild bird monitoring efforts have been undertaken in Germany and led to detection of several outbreaks of HPAI H5N1 and several infections with LPAI H5 and H7 virus in domestic poultry and wild birds [2, 7, 11]. Infections with AIV subtypes H5/H7 have been detected in wild birds and domestic poultry in Germany in close geographical proximity, but not necessarily at the same point in time (Fig. 2, Table 1). This fact alone, however, is insufficient to draw meaningful conclusions related to the sources of the outbreaks in poultry but emphasizes the complexity of the epidemiological situation [21]. Nevertheless, knowledge about the prevalence of AIV subtypes in wild birds remains pivotal for assessing the risk of AIV incursions into poultry populations [3, 20]. When reflecting upon the period of intensive wild bird monitoring, the question arises as to whether a more targeted and logistically less demanding active monitoring approach can be chosen, while at the same time maintaining the passive monitoring approach, as outlined in the EU DG SANCO guidelines [5].

We therefore investigated a scenario, where indicator species are selected in spatially confined regions

Table 3. *Distribution of haemagglutinin subtypes in German wild bird groups*

State	H subtype 5	7	3	11	6	1	4	9	2	10	8	13	12	16	Total
MV															12
BW															11
BY															11
BB															8
NW															8
RP															8
HH															5
SH															4
NI															4
BE															3
TH															3
HE															2
SN															1
ST															1
SL															1
HB															0
Total	12	6	9	8	8	7	7	7	5	5	4	2	1	1	
Groups															
Wild ducks	147	11	30	16	24	15	23	7	4	14	7		1		299
Swans	168	2	1	4	2	1		5				1			184
Wild geese	29	1		2	9	6		7	1			1			56
Grebes	289					1									299
Waders	11											1		1	13
Raptors	22									1					23
Others	7	3	6				1			5					22
Total	682	17	36	19	37	23	26	14	10	20	7	3	1	1	

BB, Brandenburg; BE, Berlin; BW, Baden-Wuerttemberg; BY, Bavaria; HB, Bremen; HE, Hesse; HH, Hamburg; MV, Mecklenburg-Western Pomerania; NI, Lower Saxony; NW, North Rhine-Westphalia; RP, Rhineland-Palatinate; SH, Schleswig-Holstein; SL, Saarland; SN, Saxony; ST, Saxony-Anhalt; TH, Thuringia.
The H subtypes are listed according to Federal State and wild bird group in Germany as identified from 1 January 2006 to 31 December 2010, in collected samples.
Dark grey shading indicates H5/H7 subtypes; light grey shading indicates H subtypes other than H5/H7.

associated with Ramsar sites. Combining information on the presence and abundance of wild bird species at Ramsar sites (Fig. 1) in Germany with the prevalence of AIV H5/H7 subtypes in those species (Figs 1, 2, Table 2) allowed us to identify a selected number of wild bird species ($n = 9$) as indicator species, representative for Germany (Fig. 1, Table 2). This approach was chosen 'due to the well-known reservoir function of wild birds (in particular of migratory waterbirds) for all LPAI subtypes' [5].

For a comprehensive monitoring approach, we propose to include two raptor species as additional targets for a focused wild bird monitoring in Germany, namely the European Buzzard and the Peregrine Falcon (Table 2). Although these species do not occur in high abundance at Ramsar sites, their numbers are high enough to qualify as indicator species according to our definition (see Materials and methods section). While the Peregrine Falcon consistently showed a percentage of $\geqslant 0.5\%$ H subtype-positive samples, the European Buzzard showed on average 0·4% H subtype-positive samples during the study period (Table 2) [10, 13, 22]. As birds of prey they may act as 'accumulators' for any H subtypes present in any of the bird species they prey on [7, 11]. We decided to apply a detection level of 0·5% of H subtype-positive samples – except for the European Buzzard – since the overall prevalence of H5/H7 subtypes in wild birds analysed as part of the active and passive wild bird monitoring in Germany during the study period was not higher than 0·85%. Therefore, establishing a threshold for a positive detection level of all

H subtypes of at least 0·5% seemed reasonable to ensure a selection of a meaningful number of wild bird species while avoiding inclusion of too many wild bird species into the category of indicator species.

The high percentage of AIV-positive samples supplied by hunted wild bird species (Table 3) may be explained by the fact that (1) a large percentage of those birds were mallards, which show the highest diversity and abundance of H subtypes among hunted birds (Table 2) and (2) their hunting season coincides with the migration of anatids in autumn, when the highest AIV prevalences were registered [23]. Since Germany is a federation, and legislation on hunting lies partly within the jurisdiction of the Federal States, there is a total of 16 Federal State hunting laws (Landesjagdgesetze) plus a federal hunting law (Bundesjagdgesetz) outlining the basic hunting rules and regulations within Germany. While federal legislation (Bundesjagdgesetz) defines and lists those wild animal species falling under the German national hunting legislation, each Federal State can add additional species and determine varying hunting seasons for its territory in its specific Landesjagdgesetz (Table 2) [17, 18]. Therefore, close collaboration with the respective hunting community constitutes a valuable asset for a successful and targeted wild bird monitoring approach, at least in Germany [17, 18].

The described method of determining indicator species for wild bird monitoring in Germany can be tailored to any wild bird wetland habitat worldwide, if information on previous avian influenza monitoring results of the wild bird species in the selected area is available. This method can then be applied to active and passive wild bird monitoring in the whole area in question, as has been applied here as an example to the whole active and passive wild bird monitoring programme in Germany. The expertise of local ornithologists will also be useful for selecting indicator species for AIV monitoring. Moreover, hunters can contribute to the monitoring efforts by supplying samples from any indicator species within a hunting season in their respective region (Tables 1–3). It has also been proposed to use sentinel birds (accessible, but fenced hand-raised Mallard ducks) as a cost-effective tool in wild bird surveillance activities [3, 24]. They should preferably be located in Ramsar sites and could contribute to virus findings in those periods of the year and in areas when and where the indicator species are not accessible (e.g. outside the hunting season).

A qualitative approach in Switzerland to measuring the effectiveness of active AIV surveillance with respect to costs concluded that active surveillance in both, wild birds and poultry, was not expected to change the probability of detection of either primary or secondary HPAI outbreaks in Switzerland [25]. The authors therefore concluded that, as a rationale of investment of resources, the estimated surveillance costs of €31 000 per year still had to reflect the value policy makers attribute to other benefits from having surveillance, for example 'peace of mind' [25].

When we applied our method to active and passive wild bird monitoring in Germany for the study period, the following picture emerged: apart from one single Mute Swan, which was actively sampled in 2007 [26], all HPAIV H5N1 cases in the study period were obtained through passive monitoring efforts; wild birds were either found dead or hunted because they showed symptoms of disease, a finding that coincides with the conclusion from the Swiss study [25].

Altogether 25 species were identified with a positive HPAIV H5N1 result. Of those, ten (40%) were also indicator species. There was at least one indicator species included with a positive identification of H5N1 in any HPAI event in wild birds in Germany. Contrary to the identification of HPAI H5N1, the detection of most LPAI subtypes was the result of active wild bird monitoring efforts [7, 8]. We therefore propose to apply an active and passive monitoring system focusing on the indicator species we identified throughout the whole of Germany.

We verified that (1) all outbreaks of HPAI H5N1 that were detected in Germany would still have been detected if we had applied our targeted method instead of the 'blanket' monitoring approach to wild bird monitoring in Germany during the study period. Apart from one animal, wild birds were submitted for laboratory testing for AIV due to the signs of disease they showed (passive monitoring) and not as part of a routine, active monitoring procedure; (2) 13 (H1–H12, H16) out of 14 (H1–H13, H16) different H subtypes, i.e. 93% including H5 and H7, would also have been detected when limiting the active monitoring to our proposed indicator species only; (3) only 29% of samples would have had to be collected, resulting at the same time in a reduction of costs for laboratory analysis of 71%. We therefore believe that this approach (preferably combined with the establishment of sentinel flocks in targeted Ramsar sites) may contribute to the objective of increasing the

efficiency of detection of avian influenza by wild bird monitoring in a cost-effective way.

APPENDIX. The BL Monitoring Group

The Group comprises of the regional diagnostic laboratories of the German Federal States of: Baden-Wuerttemberg, Bavaria, Berlin, Brandenburg, Hamburg, Hesse, Mecklenburg-Western Pomerania, Lower Saxony, North Rhine-Westphalia, Rhineland-Palatinate, Schleswig-Holstein, Saarland, Saxony, Saxony-Anhalt, Thuringia.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Alexander DJ.** An overview of the epidemiology of avian influenza. *Vaccine* 2007; **25**: 5637–5644.
2. **Schoene CUR, et al.** The puzzle on the role of wild birds in the epidemiology of highly pathogenic avian influenza [in German]. *Tierärztliche Umschau* 2009; **64**: 77–783.
3. **Knight-Jones TDJ, et al.** Evaluation of effectiveness and efficiency of wild bird surveillance for avian influenza. *Veterinary Research* 2010; **41**: 50.
4. **Kalthoff D, Globig A, Beer M.** (Highly pathogenic) avian influenza as a zoonotic agent. *Veterinary Microbiology* 2009; **140**: 237–245.
5. **European Commission.** EC-SANCO/10268/2006 – Guidelines on the implementation of survey programmes for avian influenza in poultry and wild birds to be carried out in the member states in 2007 (http://ec.europa.eu/food/animal/diseases/eradication/guidelines_SANCO_10268_2006_en.pdf). Accessed 5 January 2012.
6. **European Commission.** Decision 2005/732/EC approving the programmes for the implementation of Member States' surveys for avian influenza in poultry and wild birds. (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:274:0095:0101:EN:PDF). Accessed 2 February 2012.
7. **Globig A, et al.** Epidemiological and ornithological aspects of outbreaks of highly pathogenic avian influenza virus H5N1 of Asian lineage in wild birds in Germany, 2006 and 2007. *Transboundary Emerging Diseases* 2009; **56**: 57–72.
8. **Wilking H, et al.** Chances and limitations of wild bird monitoring for avian influenza virus H5N1 – detection of pathogens highly mobile in time and space. *PLoS ONE* 2009; **4**: e6639.
9. **Spackmann E, et al.** Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *Journal of Clinical Microbiology* 2002; **40**: 3256–3260.
10. **Wildvogelmonitoring Datenbank (AI-DB).** (https://ai-db.fli.bund.de). Restricted access. Accessed 15 December 2011.
11. **Harder TC, et al.** Bird populations – breeding grounds of pandemic influenza viruses? [in German] *Berliner und Münchener tierärztliche Wochenschrift* 2009; **122**: 440–445.
12. **Kroschewski K, et al.** Animal disease outbreak control: the use of crisis management tools. *Revue Scientifique et Technique de l'Office International des Epizooties* 2006; **25**: 211–221.
13. **Tierseuchennachrichtensystem (TSN).** (https://tsn.fli.bund.de). Restricted access. Accessed 22 June 2012.
14. **Ramsar Wetlands.** (http://ramsar.wetlands.org/Database/Searchforsites/). Accessed 17 December 2011.
15. **Ramsar.** (http://www.ramsar.org/). Accessed 17 December 2011.
16. **European Commission.** European Commission Regulation (EC) No. 1059/2003 of the European Parliament and of the Council of 26 May 2003 on the establishment of a common classification of terrestrial units for statistics (NUTS). (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:154:0001:0041:EN:PDF). Accessed 10 December 2011.
17. **Landesjagdgesetze.** (http://www.schonzeiten.de/index1.php). Accessed 18 December 2011.
18. **Bundesjagdgesetz.** (http://www.schonzeiten.de/index1.php). Accessed 18 December 2011.
19. **Pannwitz G, Wolf C, Harder T.** Active surveillance for avian influenza infection in wild birds by analysis of avian fecal samples from the environment. *Journal of Wildlife Diseases* 2009; **45**: 512–518.
20. **Snow LC, et al.** Risk-based surveillance for H5N1 avian influenza virus in wild birds in Great Britain. *Veterinary Record* 2007; **161**: 775–781.
21. **Fergus R, et al.** Migratory birds and avian flu. *Science* 2006; **2**: 845–846.
22. **IUCN Red List of Threatened Species.** Version 2011.2 (http://www.iucnredlist.org/). Accessed 19 December 2011.
23. **Munster VJ, et al.** Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. *PLoS Pathogens* 2007; **3**: e61.
24. **Globig A, et al.** Sentinel ducks as an effective tool for active avian influenza surveillance in wild birds. *Emerging Infectious Diseases* 2009; **15**: 1633–1636.

25. **Häsler B, et al.** A qualitative approach to measure the effectiveness of active avian influenza virus surveillance with respect to its cost: a case study from Switzerland. *Preventive Veterinary Medicine* 2012; **105**: 209–222.

26. **Harder T, et al.** Status of highly pathogenic avian influenza in wild birds and poultry in Germany 2007. [in German]. Tiergesundheitsjahresbericht 2007. Greifswald: Friedrich-Loeffler-Institut, 2008; pp. 53–62.

27. **OpenEpi.** (http://www.openepi.com/OE2.3/Proportion/Proportion.htm). Accessed 11 January 2012.