

Active surveillance in poultry in Poland for avian influenza subtypes H5 and H7

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A serological surveillance programme for avian influenza A virus (AIV) subtype H5 and H7 in poultry was implemented in Poland in 2008-2013 with two main objectives: i) to detect subclinical infections or previous exposures to AIV H5 and H7 subtypes and ii) to demonstrate the AI-free status of Poland. During this period, over 45 000 serum samples from 2833 holdings were examined using the hemagglutination inhibition test (HI). The presence of HI antibodies was detected in 8 breeder geese holdings (7 positive for H5 and 1 positive for H7 AIV) and in 1 breeder duck holding (H5-positive), which represented 0.32% of all investigated holdings. All seropositive flocks were examined by real time RT-PCR with negative results, which substantiated the AI-free status of Poland. Positive results detected in clinically healthy poultry kept in an open range system indicate prior infections with low pathogenic AIV originating from the wild-bird reservoir.

Key words: avian influenza virus (AIV), poultry, surveillance, hemagglutination inhibition test (HI)

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INTRODUCTION

Avian influenza viruses have been isolated from more than 100 different species of wild birds, mostly belonging to the orders: *Anseriformes* and *Charadriiformes* (Olsen *et al.*, 2006). Transmission of AIV from wild birds to poultry is a relatively frequent event, but viruses cause none or only mild symptoms and such infections are referred to as low pathogenic avian influenza. Two out of sixteen subtypes of AIV (namely H5 and H7) have the potential to mutate to a highly pathogenic avian influenza virus. Therefore, the control of AI in poultry is focused on AIV infections by any of the H5 or H7 subtypes, regardless of the virulence. The purpose of the surveillance programme implemented in the European Union is to detect subclinical or prior infections in poultry caused by AI subtypes H5 and H7 and to demonstrate the disease free status of the country (EC, 2005; EC, 2007; EC, 2010). This paper presents results of an active surveillance for AI in poultry in Poland in 2008-2013.

MATERIALS AND METHODS

Collection of samples from poultry. Blood samples for serological examination were obtained from poultry by veterinary inspection in accordance with the Commission Decision 2007/268/EC (EC, 2007) and Commission Decision 2010/367/EU (EC, 2010) and approved by the Council of Ministers and European Commission. Samples were collected in 16 Polish provinces from all species of poultry, including different types of production (with the exception of broiler chickens) and farmed game birds. The number of holdings within a province was calculated to ensure detection of at least one infected holding assuming the prevalence of infected holdings was at least 5%, with a 95% confidence interval (holdings of chickens, ostriches and game birds) or a 99% confidence interval (turkey, duck and goose holdings). The number of samples taken from ostrich flocks was 5, for laying hens, chicken breeders, turkeys and game birds it was 10, and from geese and duck flocks between 20 and 40, to ensure identification of at least one positive bird assuming the prevalence of AIV infection within a flock was about 30% with a 95% confidence interval. The larger sample size in the case of geese and ducks was dictated by lower sensitivity of the diagnostic test when used for samples collected from waterfowl (EC, 2010).

Hemagglutination inhibition test. Serum samples were examined by HI for antigens representing two AIV subtypes — H5 (H5N3) and H7 (H7N7)(AHVLA, UK). In the case of positive results, an additional test was performed with H5N1 or H7N1 antigens in order to rule out the neuraminidase cross-reactive antibodies (EC, 2010). The HI assay was carried out using 4 hemagglutinating units of the AIV antigen, according to the OIE Diagnostic Manual (2008). The cut-off titer for seropositivity was ≥ 16 ($\geq 4\log_2$). All seropositive flocks were re-tested using AI MultiS-Screen Antibody Test (IDEXX, USA) or ID Screen Influenza H5 or H7 Antibody Competition Kits (ID VET, France), according to the manufacturer's protocols. In response to detecting any seropositive flocks, follow-up investigations were carried out, including an epidemiological inquiry, clinical examination and collection of 20 oropharyngeal and 20 cloacal swabs for molecular diagnosis of AI by real time RT-PCR.

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Abbreviations: AI, avian influenza; AIV, avian influenza virus; EU, European Union; ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination inhibition test; HPAI, highly pathogenic avian influenza; LPAI, low pathogenic avian influenza; RT-PCR, reverse transcription-polymerase chain reaction.

Molecular methods of AIV identification. Total RNA was extracted from swab samples using RNeasyMini Kit (Qiagen, Germany), according to the manufacturer's protocol with some modifications. Samples were

tested by the real time RT-PCR (rRT-PCR) method, with some modification, to detect the matrix (M) gene of influenza A viruses (Spackman *et al.*, 2002).

Table 1. Results of the serological surveillance of avian influenza in poultry in Poland in 2008–2013.

Year	Poultry categories	Number of tested flocks	Number of tested samples	Number of seropositive flocks (subtype)
2008	Laying hens	82	820	0
	Chicken breeders	66	660	0
	Turkey	72	720	0
	Geese	84	3 360	0
	Ducks	8	320	0
	Farmed game birds	31	310	0
	Ratites	50	250	0
	Total:	393	6 440	0
2009	Laying hens	66	660	0
	Chicken breeders	66	660	0
	Turkey	62	620	0
	Geese	85	3 400	1 (H5)*
	Ducks	11	440	0
	Farmed game birds	36	360	0
	Ratites	52	260	0
	Total:	378	6 400	1 (H5)*
2010	Laying hens	66	660	0
	Chicken breeders	67	670	0
	Turkey	94	940	0
	Geese	85	3 400	2 (H5)*
	Ducks	13	520	0
	Farmed game birds	34	340	0
	Ratites	50	250	0
	Total:	409	6 780	2 (H5)*
2011	Laying hens	60	600	0
	Chicken breeders	64	640	0
	Turkey	95	950	0
	Geese	99	3 960	3 (H5)*
	Ducks	61	2 440	1 (H5)*
	Farmed game birds	34	340	0
	Ratites	48	240	0
	Total:	461	9 170	4 (H5)*
2012	Laying hens	91	910	0
	Free range laying hens	21	210	0
	Chicken breeders	59	590	0
	Turkey	79	790	0
	Geese	170	3 400	1 (H7)*
	Ducks	92	1 840	0
	Farmed game birds	36	360	0
	Ratites	40	200	0
Total:	588	8 300	1 (H7)*	

	Laying hens	80	800	0
	Free range laying hens	29	290	0
	Chicken breeders	66	660	0
	Turkey	64	640	0
2013	Geese	185	3 700	1 (H5)*
	Ducks	95	1 900	0
	Farmed game birds	38	380	0
	Ratites	47	235	0
	Total:	604	8 605	1 (H5)*
Total:		2 833	45 695	8 (H5)* 1 (H7)*

*in retrospective investigation (virology) indicate lack of active AIV infection

RESULTS AND DISCUSSION

The detailed results of serological examination of poultry in 2008–2013 in Poland are shown in Table 1. During the 6-year period, 45 695 samples of serum from 2 833 poultry flocks were tested, including 4 950 samples from 495 flocks of conventional and free-range laying hens, 3 880 samples from 388 flocks of chicken breeders, 4 660 samples from 466 flocks of turkeys (breeders and fattening), 28 680 samples from 988 flocks of geese and ducks (breeder and fattening), 2 090 samples from 209 flocks of farmed game birds (pheasants) and 1,435 samples from 287 flocks of ratites. The H5 subtype antibodies were found in 7 flocks of breeder geese and in 1 flock of breeder ducks. Moreover, antibodies to H7 subtype of AIV were detected in 1 flock of breeder geese. The seropositive flocks represented 0.32% of all investigated holdings. The detailed description of seropositive

flocks is provided in Table 2. All serum samples from seropositive flocks were additionally examined by the ELISA test, and the obtained results confirmed the presence of type-A specific antibodies (IDEXX AI MultiScreen Ab) and antibodies to H5 or H7 AIV subtypes (ID VET ELISA Kits).

The major objective of the active serological surveillance in the EU Member States, i.e. testing live, clinically healthy birds, is to inform competent authorities about circulating H5 and H7 strains of LPAIV in gallinaceous poultry (i.e. chickens, turkeys, guinea fowls, partridges, pheasants and quails), ratites and domestic waterfowl (i.e. geese and ducks) as well as H5 and H7 HPAIV in geese and ducks (EC, 2007; EC, 2010). Early detection of LPAIV of subtypes H5 and H7 in poultry can expedite the implementation of control measures in order to prevent its mutation into a HPAI virus. Active surveillance for HPAIV in geese and ducks aims at detecting



Figure 1. The distribution of seropositive holdings tested during the survey for avian influenza infections in Poland in 2008–2013.

Table 2. Seropositive poultry flocks.

Year	Code of flock	Poultry category	Age (year)	AIV subtype	% of seropositivity			Region
					HI	ELISA AI A (IDEXX)	ELISA H5/H7 (ID VET)	
2009	M044/09	breeder geese	4	H5	25.0%	100.0%	52.5%	Warmińsko-Mazurskie
2010	M082/10	breeder geese	3	H5	50.0%	57.5%	82.5%	Wielkopolskie
2010	M175/10	breeder geese	2	H5	40.0%	57.5%	100.0%	Kujawsko-Pomorskie
2011	M071/11	breeder ducks	1	H5	25.0%	100.0%	75.0%	Wielkopolskie
2011	M182/11	breeder geese	4	H5	27.5%	100.0%	67.5%	Warmińsko-Mazurskie
2011	M334/11	breeder geese	3	H5	42.5%	27.5%	70.0%	Wielkopolskie
2011	M362/11	breeder geese	3	H5	35.0%	22.5%	65.0%	Lubelskie
2012	M232/12	breeder geese	4	H7	40.0%	100.0%	85.0%	Kujawsko-Pomorskie
2013	M149/13	breeder geese	–	H5	10.0%	75.0%	60.0%	Małopolskie

subclinical infections since domestic waterfowl, mostly ducks, exhibit a range of clinical outcomes, including asymptomatic infections (Pantin-Jackwood & Swayne, 2007; Phuong do *et al.*, 2011).

The serosurvey conducted in Poland has indicated a lack of active infections with avian influenza viruses in the poultry population and a low percentage of seropositive holdings (0.32%). There were no virus positive results in industrial poultry farms. All positive flocks were waterfowl, mostly breeder geese aged 1–4 years (median age 3 years), kept in an open-range system at least in some periods of their life and which might have had, at least temporarily, a close contact with wild birds representing the potential source of infection (Olsen *et al.*, 2006). Epidemiological investigation in seropositive flocks did not reveal any health disorders, including breeding problems, during the entire lifespan. Our results suggest that seropositive birds were exposed to infection with low pathogenic AIV of wild-bird origin rather than a highly pathogenic AI virus (mostly H5N1 HPAIV) for two reasons. Firstly, an open-range system, in which domestic waterfowl are kept, favours frequent opportunities of direct and indirect contacts with wild birds and the circulation of both H5 and H7 LPAIV in the wild reservoir has been confirmed in recent years during active surveillance studies carried out in Poland (Smietanka *et al.*, 2011; Smietanka *et al.*, 2012). Moreover, the majority of seropositive holdings were located in regions abundant with lakes, such as the Masurian Lake District, the Great Poland Lakeland and the Łęczna-Włodawa Lakeland (Fig. 1), in which wild waterfowl form massive aggregations in certain seasons. Secondly, although the HI test does not allow for the identification of the neuraminidase subtype, the detailed analysis of all positive results revealed lower titers of antibodies against the secondary H5N1 antigen than the primary H5N3 antigen (data not shown).

The results of the surveillance in Poland are similar to those obtained for the whole EU (annual reports published at [\[trolmeasures/avian/eu_resp_surveillance_en.htm\]\(http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/eu_resp_surveillance_en.htm\)\). The slightly higher detection rate noted in Poland \(0.32%\) in comparison to the EU as a whole \(0.21%\) was most likely related to the higher proportion of examined geese and duck holdings \(high-risk species\), which constituted 34.9% of the total sampled poultry holdings in Poland. Likewise as in Poland, the highest apparent prevalence for H5 was observed in ducks and geese in other Member States. Seroreagents to H7 subtype were found in Europe mainly in backyard flocks and conventional as well as free-range laying hens.](http://ec.europa.eu/food/animal/diseases/con-</p>
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To summarize, results of the conducted studies indicate that infections with identifiable AI of H5 and H7 subtypes in the poultry population in Poland are sporadic and most probably result from the incursions of avian influenza viruses of wild-bird origin.

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