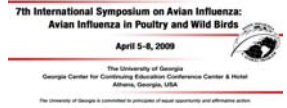


Serological Testing for Avian Influenza Viruses in Wild Birds: Comparison of Two Commercial Competition ELISAs

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INTRODUCTION

The H5N1 epidemic has resulted in commitment of resources towards improving avian influenza virus detection tools, especially in wild birds (Charlton *et al.* 2008). AGID, HI test and indirect ELISA have been used in serologic diagnostic in poultry. Nevertheless, some constraints exist in relation to serologic testing in wild birds. AGID test cannot be used due to the lack of precipitins, HI gives information only about specific HA subtypes, has a relatively low sensitivity in some wild species such as ducks (Starick *et al.* 2006) and is expensive and labor intensive. More recently, competitive ELISA systems (cELISA) have been implemented to detect antibodies against AI NP in different avian species (Shafer *et al.* 1998, Starick *et al.* 2006). This refined species independent approach has been used for wildlife surveillance (de Marco *et al.* 2003), and is now commercially available. The aim of this study is to compare results from two commercial cELISA using wild bird sera from different species and assessing usefulness of this kind of assays in wild bird AIV surveillance.

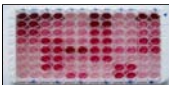


MATERIALS AND METHODS

In our study we employed samples from a plasma bank collected between 1995 to 2007 from birds of prey, storks, sentinel ducks and other waterbirds admitted to rehabilitation centres in Castilla-La Mancha in south central Spain. A total of 1400 plasma samples were tested against H5/H7 subtype AIV using HI test with 4 haemagglutinating units as described by OIE, 2008. A subset of 96 plasma samples from 23 species were selected (Table 1) and analysed blindly using two different ELISAs (ELISA1 Ingezim Influenza A®, Ingenasa, Spain; ELISA2 IDscreen, influenza A antibody competition®, idvet, France). Both ELISAs are based on a competition protocol and are supposed to be applicable to multiple species. Samples that tested positive in one of the ELISAs but negative in the HI test against H5 and H7 were subsequently tested by HI against H1, H2, H3, H6, H9, H10 and H12.

Table 1

FAMILY	SPECIES	N
Accipitridae N=45	Spanish Imperial Eagle (<i>Aquila adalberti</i>)	10
	Golden eagle (<i>Aquila chrysaetos</i>)	7
	Marsh harrier (<i>Circus aeruginosus</i>)	6
	Eurasian black vulture (<i>Aegypius monachus</i>)	5
	Black kite (<i>Milvus migrans</i>)	5
	Red Kite (<i>Milvus milvus</i>)	4
	Common buzzard (<i>Buteo buteo</i>)	3
Anatidae N=88	Gadwall (<i>Anas strepera</i>)	1
	Northern Pintail (<i>Anas acuta</i>)	1
	Mallard (<i>Anas platyrhynchos</i>)	6
	Sentinel duck (<i>Anas sp.</i>)	??
	Greylag geese (<i>Anser anser</i>)	1
	<i>Anas sp.</i>	1
	Red-crested pochard (<i>Netta rufina</i>)	1
Ardeidae N=2	Purple heron (<i>Ardea purpurea</i>)	1
	Grey Heron (<i>Ardea cinerea</i>)	1
Ciconiidae N=16	White stork (<i>Ciconia ciconia</i>)	16
Corvidae N=4	Maggie (<i>Pica pica</i>)	4
Laridae N=3	<i>Larus sp.</i>	3
Phalacrocoracidae N=2	Great cormorant (<i>Phalacrocorax carbo</i>)	2
Phasianidae N=2	Red-legged partridge (<i>Alectoris rufa</i>)	2
Phoenicopteridae N=1	Greater flamingo (<i>Phoenicopterus ruber</i>)	1
Strigidae N=8	Eurasian Eagle-owl (<i>Bubo bubo</i>)	8
TOTAL		171



RESULTS

Of 22 samples that tested positive against H5 or H7 AIV subtypes (HI titer $\geq 1:16$), 15 were detected by ELISA 1 (14 positive, 1 doubtful), and 8 by ELISA 2 (6 positive, 2 doubtful) (Table 2). Using the HI as gold standard, and including ELISA positive results with the cut-off defined by the manufacturer, sensitivity for the detection of H5/H7 AIV antibodies was 68.2% and 36% respectively for ELISA1 and ELISA2. 45 additional samples were positive by ELISA1 (17 doubtful) and 31 (14 doubtful) by ELISA2 but negative by HI H5/H7. None of the ELISA-positive/HI(H5/H7) negative samples tested against additional hemagglutinins (n = 43) gave a positive result. Due to the reduced amount of sample volume it was not possible to test the samples against all HA subtypes (H4, H8, H11, H13, H14, H15, H16 were not tested). Distribution of results among families is shown in Figure 1.



Figure 1

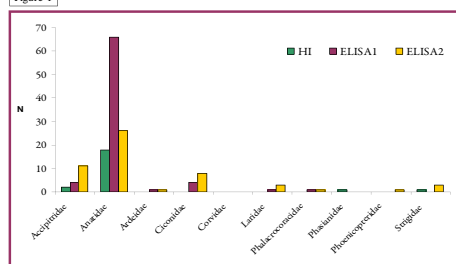


Table 2

Family	N	HI H5/H7+	ELISA1+		ELISA2+	
			HI H5/H7+	HI H5/H7-	HI H5/H7+	HI H5/H7-
Accipitridae	45	2	1	3	2	9
Anatidae	88	18	14	52	5	21
Ardeidae	2	0	0	1	0	1
Ciconiidae	16	0	0	4	0	8
Corvidae	4	0	0	0	0	0
Laridae	3	0	0	1	0	3
Phalacrocoracidae	2	0	0	1	0	1
Phasianidae	2	1	0	0	0	0
Phoenicopteridae	1	0	0	0	0	1
Strigidae	8	1	0	0	1	2



DISCUSSION

Our study assesses the usefulness of recently available commercial multispecies ELISA kits for the analysis of AIV seroprevalence in multiple wild bird species. ELISAs have been previously used in wild birds in Spain (Arenas *et al.*, 1990; Astorga *et al.*, 1994), Italy (de Marco *et al.* 2003) and Germany (Starick *et al.* 2006), but in all cases the ELISAs employed were in-house and are not commercially available.

In our study, numerous samples tested positive by one or both of the ELISAs but were negative by HI against H5 and H7 and an additional 7 HA subtypes tested (Table 2). These results may mean presence of antibodies against any of the HA subtypes that were not tested, but also non-specific reactions of the samples in the test system. Alternatively it could reflect a higher sensitivity of the cELISAs as compared to HI as stated by other authors, due to the considerable variation in the immune response among avian species. As an example, antibodies may be induced in ducks that are not precipitating and cannot be detected in conventional HI test.

Performance of ELISA 1 appears to be comparable to the test used by De Marco *et al.* (2004) and Starick *et al.* (2006) with the drawback that this test requires a very high amount of sample (50µl), while ELISA 2 is configured for a very small amount of sample (10µl) which may negatively affect performance and need adjustment.

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