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 (cELISAs) 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 cELISAs using wild bird sera from different species and assessing usefulness of this kind of assays in wild bird AI 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 AI V 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 (ELISA 1: Ingezim Influenza A®, Ingenasa, Spain; ELISA 2: 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.

RESULTS

Of 22 samples that tested positive against H5 or H7 AI V subtypes (HI titer ≥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 AI V antibodies was 68.2% and 36% respectively for ELISA 1 and ELISA 2. 45 additional samples were positive by ELISA 1 (17 doubtful) and 31 (14 doubtful) by ELISA 2 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.

DISCUSSION

Our study assesses the usefulness of recently available commercial multispecies ELISA kits for the analysis of AI V seroprevalence in multiple wild bird species. ELISAs have been previously used in wild birds in Spain (Arenas et al., 1990, Auterga et al., 1994a), 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.

REFERENCES