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Antibody survey on avian influenza viruses using egg yolks of ducks in Hanoi between 2010 and 2012

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ABSTRACT

In Vietnam, numerous surveillance programs are conducted to monitor the prevalence of avian influenza (AI) viruses. Three serological methods—the agar-gel immunodiffusion test, hemagglutination inhibition (HI) test, and enzyme-linked immunosorbent assay—are well established for detection of AI virus antibodies in poultry sera. Several recent reports have validated egg yolk as an alternative source for detection of AI virus antibodies. In this study, we investigated AI virus antibodies in ducks by HI testing using egg yolk. Ten duck eggs were collected every month from 10 randomly selected markets in Hanoi from April 2010 to March 2012. The HI test was performed using low pathogenic avian influenza (LPAI) viruses (H3, H4, H6, H7, H9, and H11 subtypes) and highly pathogenic avian influenza (HPAI) viruses (H5N1 clade 2.3.4 and 2.3.2.1) as antigens. HI testing for H3, H6, and H9 was 29% positive in November 2010, 50% positive in October and November 2010, and 12% positive in June 2011. These results indicated that several epidemics of LPAI viruses had occurred during the study period. In addition, antibodies against H7 were negative. The results of HI testing for H5N1 showed that the reactivity of the dominant HI antibody shifted from H5N1 clade 2.3.4 to clade 2.3.2.1. In conclusion, egg yolk is useful for long term monitoring of AI virus antibodies and the use of egg-based antibody detection may contribute to improvements in animal welfare.

1. Introduction

Outbreaks of highly pathogenic avian influenza (HPAI) of the H5N1 subtype have occurred in Vietnam since December 2003 (Hien et al., 2009). Ducks are of particular concern because they are asymptomatic carriers of avian influenza (AI) viruses including low pathogenic avian influenza (LPAI) and HPAI (Chen et al., 2004; Sturm-Ramirez et al., 2005). Therefore, ducks play an important role in transmission of AI viruses. Recently, various AI viruses were isolated from ducks in Vietnam, including the H3N2, H3N8, H4N6, H5N1, H5N2, H6N1, H9N2, H9N3, H9N6, H11N3, and H11N9 subtypes (Hotta et al., 2012; Nguyen et al., 2005; Nomura et al., 2012).

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In 1997, an H5N1 influenza virus outbreak occurred among chickens in Hong Kong, and the virus was transmitted directly to humans. Phylogenetic analysis indicated the highest homology between the internal genes of A/quail/Hong Kong/G1/97 (H9N2), A/teal/Hong Kong/W312/97 (H6N1), and the H5N1 isolates (Guan et al., 1999; Hoffmann et al., 2000). These reports suggest that reassortment occurred between the H9N2, H6N1, and H5N1 viruses, possibility involving the internal genes of the H5N1 virus, which were acquired from H9N2 and H6N1. To control HPAI viruses and monitor the generation of novel viruses, surveillance for AI viruses among poultry is needed in countries where H5N1 strains are circulating.

Sero-epidemiological studies targeting a specific antibody against AI viruses are commonly used to collect evidence of infection or to evaluate the effects of vaccination. Because animal welfare is an issue of great concern, there is a requirement for alternative sources of antibodies that can be produced without pain and distress to the animals (Silim and Venne, 1989). In terms of animal welfare as well as economic considerations, the use of egg yolk antibodies instead of serum is sufficient to allow AI surveillance among chickens and ducks (Beck et al., 2003; Jeong et al., 2010; Trampel et al., 2006).

In this study, we examined an egg yolk antibody as an alternative source for AI virus antibody detection in layer ducks, and antibodies against hemagglutinin (HA) were used as markers for both infection and vaccination. Because the vaccine used in northern Vietnam is generated from a genetically modified reassortant H5N1 virus, differentiation between the virus in infected and vaccinated poultry is difficult when measuring the antibody response against HA. To monitor the prevalence of AI viruses in ducks, we collected duck eggs from markets in Hanoi, and examined hemagglutination inhibition (HI) antibodies using LPAI viruses as antigens. In addition, to investigate whether the reactivity of HI antibodies was different between different clades of H5N1 viruses, we performed HI testing using HPAI H5N1 clade 2.3.4 and 2.3.2.1 viruses as antigens.

2. Materials and methods

2.1. Sample collection and preparation of egg yolk

In total, 2378 duck eggs were collected in Hanoi from April 2010 to March 2012. Ten eggs were obtained from each of the 10 randomly selected markets every month to yield 100 eggs. For yolk immunoglobulin extraction using a simplified chloroform polyethylene-glycol procedure, 2 ml of egg yolk was mixed with an equal volume of phosphate-buffered saline, and then added to 4 ml of chloroform (Polson, 1993). After mixing well, the yolk was centrifuged at 3500 rpm for 10 min. The supernatant was collected and used for antibody tests.

2.2. Virus and antigen preparation

LPAI A/duck/Ukraine/1/63 (H3N8), A/duck/Czechoslovakia/1/56 (H4N6), A/turkey/Massachusetts/3740/65 (H6N2), A/whistling swan/Shimane/35/80 (H6N6), A/whistling swan/Shimane/42/80 (H7N7), A/swan/Shimane/42/99 (H7N8), A/turkey/Massachusetts/3740/65 (H9N2), A/whistling swan/Shimane/48/97 (H11N2), A/duck/England/1/56 (H11N6), HPAI A/Vietnam/31244/2007 (H5N1, clade 2.3.4), A/muscovy duck/Vietnam/LBM57/2011 (H5N1, clade 2.3.2.1), and swine influenza A/swine/Iowa/15/30 (H1N1) were propagated in 9–10-day-old embryonated chicken eggs. Before using the embryonated eggs, HA testing was performed to confirm that the eggs did not contain antibodies against influenza viruses. Viruses in the harvested allantoic fluids were inactivated with 0.1% formalin (v/v) for 7 days at 4 °C. Virus inactivation was confirmed by 2 blind passages in embryonated eggs.

2.3. HI test

The HI test was performed according to the standard procedures recommended by the World Health Organization. Briefly, the yolk samples were treated with a receptor-destroying enzyme (RDE) (Denka Seiken Co. Ltd., Tokyo, Japan) at 37 °C for 20 h to eliminate non-specific inhibitors of hemagglutination. HI titers obtained from a purified yolk and RDE mixture (25 μl egg yolk + 75 μl RDE provided a starting dilution of 1:1) were defined as the reciprocal of the highest dilution of yolk, which completely inhibited hemagglutination of 4 hemagglutination units of the virus with a 0.5% solution of chicken red blood cells. Samples with HI titers under 16 were considered negative.

3. Results and discussion

To determine the prevalence of AI viruses among ducks in Hanoi, HI testing was performed using 2378 egg yolks obtained from April 2010 to March 2012. As shown in Table 1, selected samples from the 2378 egg yolks showed patterns of positive results in the HI test. To confirm the effects of the NA subtype on HI testing, the HI test was performed using H1N1 for H5N1, H7N8 for H3N8, and H11N2 for H6N2 and H9N2. The positive samples did not overlap between H4N6 and H6N6. These results indicated that there was no effect of the NA subtype on HI testing in this study.

From April 2010 to March 2012, the positivity rates of the yolk antibody against LPAI viruses were 1.98% and 7.7% for H3 and H6, respectively. The other subtypes showed lower than 1% positivity (0.25%, 0.42%, and 0.67% for H4, H9, and H11, respectively) (Table 2). An antibody against H7 was not detected in egg yolk. H6 (7.7%) was the most frequently detected HA subtype in ducks.

As shown in Fig. 1, several epidemics of LPAI viruses were observed during the monitoring period. The HI antibody against H3 was 29% positive in November 2010, whereas the HI antibody H11 was 12% positive in June 2011. The HI antibody against the H6 subtype was detected from April 2010 to January 2011, except in August 2010. There was a drastic peak in October and November 2010 during which the yolk-antibody positivity rate was 50%. Thus far, no vaccination against LPAI viruses has been conducted for domestic poultry in Vietnam. These results