



Measurement of Systemic and Mucosal Immune Responses in Ducks after Experimental Infection with H5N1 and H7N1 LPAI Viruses



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Introduction and Objectives

- The role of wild ducks as vectors of avian influenza viruses (AIV) is well known but the immune response induced by AIV has different patterns according to the species of ducks.
- The present study aimed to develop 5 indirect ELISAs for detection of specific duck immunoglobulins classes [IgA, IgM, IgY, Ig light chain, IgY Heavy chain] to avian influenza viruses. The newly developed ELISAs were used to evaluate the systemic and local (duodenum) immunological response induced by H5N1 and H7N1 low pathogenic avian influenza (LPAI) viruses in ducks.

Materials and methods

Study I

8 23-days-old Pekin ducklings were infected with an H5N1 LPAI of duck origin at 10e6 EID50 by ED/IN route (0dpi) and 8 ducklings were mock infected as negative control. Cloacal and the oral were collected at 4 and 7dpi, blood and duodenum at 7dpi.

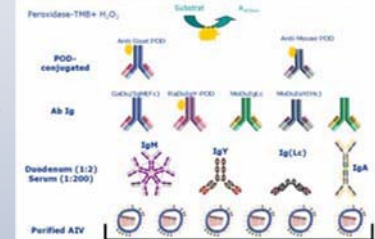
Study II

16 7-days-old Mule ducks were infected with an H7N1 LPAI of chicken origin at 10e6 EID50 by ED/IN route (0dpi) and boosted at 21dpi. 16 ducks were used as control group. Cloacal and the oral were collected at 3 and 6 dpi. Blood and duodenum were collected on a weekly basis during 4 weeks, blood was also collected at 7dpi.

Measurement of the humoral response



AIV-specific duck IgY, IgY(Hc), Ig(Lc) IgM, and IgA ELISAs



Results and Discussion

Study I

- AIV shedding:** Following infection with H5N1 LPAI, all birds were detected to be positive at 4 and 7dpi in oral and cloacal swabs by RRT-PCR (Table 1). H5N1 LPAI virus of duck origin replicates well in the gastro-intestinal tract as well as in respiratory tract in Pekin ducks.

Table 1: AIV shedding was measured by RRT-PCR targeting the matrix gene in the oral and cloacal swabs after H5N1 LPAI infection.

DPI	Oral swabs			Cloacal swabs		
	Positive	Mean Ct values	St Dev	Positive	Mean Ct values	St Dev
4	8/8 (100%)	31.65 ±2.33		8/8 (100%)	27.54 ±2.25	
7	6/8 (75%)	35.59 ±1.61		8/8 (100%)	27.29 ±5.86	

- Systemic response:** No H5N1 LPAI-specific antibody could be detected by HI test and commercial NP competitive ELISA at 7dpi. Conversely, AIV-specific antibodies could be successfully detected by total IgY, IgLc, IgY (Hc), IgM, and IgA-specific ELISA at 7dpi (Fig. 1).

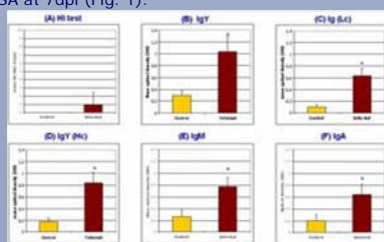


Figure 1: Systemic response 7 days after infection in sera of H5N1 LPAI infected and non infected control birds. (A) HI test, (B) AIV-specific IgY, (C) IgLc, (D) IgY (Hc), (E) IgM, and (F) IgA ELISA. * indicates a significant difference (p<0.05).

- Mucosal response:** As for the mucosal response, IgLc, IgA and IgM were detected by AIV-specific ELISA in duodenum at 7dpi; whereas no AIV-specific IgY, and IgY(Hc) were detected in duodenum at 7dpi (Fig. 2). The polymeric immunoglobulin receptor (pIgR) transports secreted IgA and IgM from intestinal villi through to the luminal side of the mucosa.

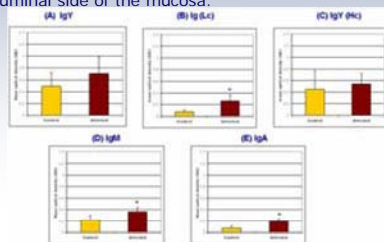


Figure 2: H5N1 specific mucosal antibody responses. (A) IgY, (B) IgLc, (C) IgY (Hc), (D) IgM, and (E) IgA ELISA in duodenum. * indicates a significant difference (p<0.05)

Study II

- AIV shedding:** Viral excretion analysis showed that RRT-PCR were positive in oral swabs at 3dpi, indicating the virus replication. On the other hand, no virus could be detected at 6dpi in oral and cloacal swabs (table 2).

Table 2: AIV detection was done by RRT-PCR (matrix gene) in the oral and cloacal swabs following H7N1 LPAI infection.

DPI	Oral swabs			Cloacal swabs		
	Positive	Mean Ct values	St Dev	Positive	Mean Ct values	St Dev
3	16/17 (94%)	26.95	±3.06	0/17 (0%)		
6	0/17 (0%)			0/17 (0%)		
10	0/17 (0%)					

- Systemic response:** AIV-specific responses were detected at 21-35dpi by the HI test. All infected ducks were positive by NP competitive ELISA at 7 to 35 dpi; whereas control ducks were negative. The AIV-specific duck IgY, IgLc, IgY(Hc), IgM, and IgA ELISAs could detect the duck immunoglobulins in sera (Fig. 3).

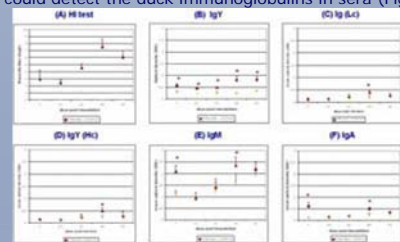


Figure 3: H7N1 specific systemic antibody responses in serum after prime-boost H7N1 LPAI infection at 0 and 21 days. AIV specific Igs were detected by HI test (A) and AIV-specific duck (B) IgY, (C) IgLc, (D) IgY (Hc), (E) IgM, and (F) IgA ELISA. * indicates a significant difference (p<0.05).

- Mucosal response:** AIV-specific IgY antibodies were detected at 21-35 dpi in duodenum. After the boost with H7N1 virus, AIV-specific duck IgA- IgM, IgLc, IgY(Hc) were also detected in duodenum at 28dpi. The detected IgY in duodenum could originate from serum by transudation, since IgY can migrate from serum through the lamina propria in chickens.



Figure 4: AIV-specific (A) IgY, (B) IgLc, (C) IgY (Hc), (D) IgM, and (E) IgA mucosal response in duodenum after prime-boost H7N1 LPAI infection at 0 and 21 days by ELISA test. *p<0.05

Conclusions

- The developed AIV-specific duck IgY, IgLc, IgY(Hc), IgM, and IgA ELISAs proved to detect efficiently the systemic and mucosal responses elicited by AIV in two different species of duck.
- To our knowledge, this is the first time that specific IgA could be detected in sera of ducks.

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