Measurement of Antibody Titer to Fowl Pox Virus by Enzyme-Linked Immunosorbent Assay

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ABSTRACT. The usefulness of the measurement of antibody titer to fowl pox virus (FPV) by enzyme-linked immunosorbent assay (ELISA) was evaluated in SPF chickens with or without inoculation with FPV. The optimum concentration of purified antigen was 10 $\mu\text{g/ml}$ of protein. The absorbance at 492 nm was less than 0.10 in the chickens negative to FPV from 1 to 63 days old. By contrast, a higher titer was detected in SPF chickens with various FPVs inoculated into the wing web than in non-inoculated chickens. Moreover, there was no cross response to chicken sera immunized with *Haemophilus paragallinarum*, Marek's disease virus, Newcastle disease virus or infectious bronchitis virus. The titers increased after vaccination were not increased after subsequent challenge with virulent FPV. These findings suggested the usefulness of the measurement of the antibody response to FPV vaccine by ELISA.—**KEY WORDS:** ELISA, fowl pox virus.

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Fowl pox is a common viral disease of chickens. This disease has long prevailed throughout Japanese poultry farms, and the fowl pox vaccine has been widely used to prevent the disease, but there are no suitable serological methods to evaluate the vaccine at present. Antibodies to fowl pox virus (FPV) can be detected by immunodiffusion [6], passive hemagglutination [3] and neutralization tests [9], but passive hemagglutination and immunodiffusion tests can be used only for detection within a short time, and the neutralization procedure is not practical as a routine diagnostic test.

The enzyme-linked immunosorbent assay (ELISA) can be used to detect FPV antibodies [1, 8], but the limitations of the positive and negative ranges of ELISA to FPV, the responsiveness of positive sera to various strains of FPV and the cross reaction between FPV and immune sera for other infectious diseases have not been reported, and must be examined before the ELISA test can be used routinely.

In the present study, we examined whether ELISA can be used as a routine diagnostic test to measure the antibody titer to FPV in chickens.

To determine the optimum concentration of the ELISA antigen, pooled sera from 10 SPF chickens with or without inoculation by the Nishigahara strain of FPV into the wing web were used as positive and negative references, respectively. Sera from 10 chicken each, from 1 day old to 63 days old were used to determine the range of negative reaction to the FPV antibody in the ELISA test. The SPF chickens from our laboratory flocks reared under isolation were used. They had not been vaccinated against any disease [5].

FPV strain USDA vaccine and virulent USC strains were obtained from Dr. K. Nazerian [10]. Vaccine strains of NP, H, #946 and NM, and virulent strains of Nishigahara, Shisui, K-59 and Tamagawa were also used for this study.

The USDA strain of FPV, which showed good growth in chicken embryo fibroblast (CEF) cell culture, was used for the antigen preparation for ELISA. Primary CEF cells were cultivated in a rolled bottle with slow rotation and then the USDA strain was incubated at 37°C for 72 hr. The cultured fluid was centrifuged at $2,000 \times g$ for 10 min

after freezing and thawing twice.

The supernatant was centrifuged at $70,000 \times g$ for 2 hr. The pellet was resuspended in 1 mM Tris-HCl buffer and then centrifuged into a linear gradient from 20 to 50% sucrose at $33,000 \times g$ for 45 min. The white band was collected and then dissolved in Dulbecco's phosphate buffer saline (PBS). After washing twice by centrifugation at $59,000 \times g$ for 30 min, the purified antigen was resuspended in PBS at a concentration one to 100 times of that the culture fluid. The concentration of protein was measured with a protein assay kit (Bio-Rad).

The procedure for ELISA was essentially the same as the general method described elsewhere [4]. Briefly, the chicken serum was inactivated at 56°C for 30 min before use. Then 0.1 ml of sera, diluted at 1 to 100 in 3% bovine serum albumin in 0.5 M NaCl containing 0.1% Tween 20, was added to each plate well which was fixed with the purified antigen. The plates were incubated at room temperature for 2 hr, and then diluted rabbit anti-chicken IgG peroxidase conjugate (Sigma) was added to each well. The substrate was added to each well after removal of the excess conjugate and the reaction was stopped with 2.5 M H_2SO_4 . The ELISA titer was expressed as absorbance at 492 nm. The optimum concentration of the antigen used in ELISA was tested in sera positive or negative to FPV. When a low concentration of antigen was used in the positive serum, the ELISA titer was deviated from the actual concentration of the antigen. The titers were not affected at a concentration of more than 10 $\mu\text{g/ml}$, but there the antigen concentration had no effect in the negative serum (Fig. 1).

When the ELISA was tested in SPF chickens with 10 $\mu\text{g/ml}$ of the purified FPV antigen, the titers in all chickens from 1 to 63 days old were less than 0.1 (Table 1). The low titer in negative chickens clearly suggested the usefulness of the ELISA test.

One-day-old SPF chickens were inoculated into the wing web with various FPV at 1×10^4 PFU per chicken. Table 2 shows the antibody titer at 3 weeks after the inoculation. The antibody titer was much higher in the chicken inoculated with various FPVs than in the non-inoculated chicken, but the titer differed somewhat among the FPV strains. This difference might be reflected in the

Fig. 1. Optimum concentration of FPV antigen. (○): Serum from FPV infected chicken, 100-fold dilution. (●): Serum from uninfected SPF chickens, 100-fold dilution.

Table 1. ELISA titer in SPF chickens without vaccination against fowl pox

Age in days	Absorbance	Age in days	Absorbance
1	0.07±0.03	35	0.05±0.01
7	0.05±0.03	42	0.04±0.02
14	0.05±0.02	49	0.06±0.02
21	0.09±0.05	56	0.09±0.04
28	0.04±0.02	63	0.06±0.01

antigenic difference among FPV strains and the variation in the growth of FPV in the chickens. The absorbance in chickens immunized with infectious diseases was 0.07 for infectious bronchitis virus, 0.10 for Newcastle disease virus, 0.09 for Marek's disease virus and 0.08 for *Haemophilus paragallinarum*. There was no cross antibody response between FPV and other infectious diseases. All 5 chickens vaccinated into the wing web with the USDA strain of FPV vaccine at 10 days old developed a lesion at the vaccination site, but no lesion was found at the challenged site after subsequent challenge with a virulent Nishigahara strain. Figure 2 shows the mean titer to FPV antibody in 5 chickens. The titer was increased at 1 week after vaccination and reached a peak at 3 weeks, but the titer did not increase after subsequent challenge. The present findings suggested the usefulness of measurement of the antibody titer to FPV by the ELISA test.

Fig. 2. Antibody response to ELISA in chickens after vaccination and subsequent challenge with fowl pox virus.

Table 2. Antibody titer in chicken inoculated with various fowl pox viruses by ELISA

FPV Strain	Absorbance in individual chickens				
	1	2	3	4	5
Shisui	0.91	1.01	1.01	0.97	1.05
Tamagawa	1.94	>2.00	>2.00	1.41	1.98
K-59	1.44	1.94	0.62	0.55	1.14
Nishigahara	1.99	1.90	1.80	1.90	1.82
USC	1.06	1.11	1.01	0.86	0.99
NP	0.79	0.65	1.15	1.06	0.79
USDA	1.05	1.63	1.23	1.50	1.77
H	0.36	0.35	0.27	0.26	0.23
#946	0.30	0.29	0.21	0.20	0.19
NM	0.73	0.66	0.44	0.36	0.35
Control	0.04	0.06	0.06	0.04	0.07

The limitations to a negative and positive response to FPV were determined to be less than 0.1 and more than 0.2 at 492 nm, respectively. Generally the parents of conventional chicks had a maternal antibody to various infectious diseases including FPV transferred to them from their parents. Further studies on conventional chicks are necessary. Experiments are now in progress on the possibility of the routine use of ELISA for the conventional flocks as reported in Newcastle disease [6].

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