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Serological Survey of Major Avian Viral Diseases Related with Egg Production in Commercial Chicken Flocks in Korea

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ABSTRACT While use of mass rearing systems improved poultry production, chances of exposing to contagious diseases have been increased, making flocks more vulnerable to diseases. Diseases of interest which affects egg production adversely include Low pathogenic avian influenza (LPAI), Infectious bronchitis (IB), Avian meta-pneumoviral infection (aMPV) and Egg drop syndrome'76 (EDS'76). This report collected and analyzed 5,385 serum samples, which were collected from 1,330 different chicken flock, provided by Chungbuk National University, Avian Disease Laboratory at 2009. Serums were analyzed based on rearing stages; 0～1.3weeks (wks) (maternal antibody period), >1.3～3 wks (starting period), >3～10 wks (growing period), >10～22 wks (developing period), >22～40 wks (peak laying period), >40～60 wks (late laying period) and over 60 wks (post-molting period). Results showed the 99.7% of the tested flocks were immunized against ND and73.8%, 97.1%, 78,2% and 78% of the flocks were immunized against other 4 agents (LPAI, IB, EDS'76, aMPV). Maternal antibody was transferred to enough quantity for NDV. Generally, antibody titers which were developed at 22 weeks were stabilized permanently for life. In case of IB and aMPV, infection titer emerged as early as 10 weeks and the titer was increased from 99.4% to 100% for life. EDS76 showed increase in titers, reflecting decreased frequency of vaccination programs. Overall, this study displayed general trends of major viral disease in layers, but considering the trend of development of preventive measures and evolution of pathogens, conducting serological surveys on a regular basis is important.

(Key words : serological survey, avian influenza, infectious bronchitis, avian metapenumovirus, commercial layer)

INTRODUCTION

High-density livestock rearing systems largely improved poultry production of production performance in poultry industry, but made the flocks more vulnerable to contagious diseases (Alexander, 2007; Capua and Marangon, 2000; Linaker and Smedley, 2002). In order to manage effective mass production systems, it is strongly required to invest heavily in disease controlling/ surveillance system.

In layer and breeder flocks, which chickens are periodically bred for a long time, serologic tests are actively conducted to monitor flock health and to evaluate the efficacy of vaccine programs (Saif et al., 2011; Sharma, 1999). Diseases which have negative effect on egg production and requiring extensive management include: Low pathogenic avian influenza (LPAI), Infectious bronchitis (IB), Avian meta-pneumoviral infection and Egg drop syndrome' 76 (EDS'76) (Lee et al., 2010; Mo et al., 2003; McFerran, 1979; Song et al., 1998; Sugiyama et al., 2006). On the other hand, the diseases which affects the immune system such as Chicken infectious anemia (CIA) and Infectious bursal disease (IBD) are also regarded as key diseases for monitoring because of their ability to undermine vaccine efficacy (Boer et al., 1994; Lee et al., 2010; Kibenge et al., 1988; Otaaki et al., 1988; Otaki et al., 1988). Despite the abundance of serologic tests, only limited comprehensive analysis of the results has been conducted. The majority of the serologic surveys in Korea have been conducted on breeder focusing on trans-ovarian transmission disease such as salmonellosis or mycoplasmosis (Lee et al., 2010). In case of layers, despite their importance layers in Korean poultry industry, serologic studies on layers have been limited even compared to breeders. Thus previous serologic studies conducted on layers were limited to a mere part of poultry industry (Lee et al., 2003; Pak, 2009; Woo and Park, 2008) not including detailed information of change of antibody titer (Pak, 2009). This discrepancy seems to occur from the absence of effective management of serologic data and well-established criteria. Recently, studies on major viral diseases

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in layers using serologic survey methods were published (Lee et al., 2010). This study analyzed 5,385 serum samples collected from 1,330 different flocks which were submitted to Avian Disease Laboratory of Chungbuk National University (CBNU-ADL, Cheongju, Korea) at 2009. The change in antibody titers was analyzed according to ages and disease.

However, continuous serologic survey is critical due to change in condition and disease patterns. The aim of this study is to analyze data of serologic tests received by CBNU-ADL at 2010. Comparison with data from previous years was followed after analysis of serologic results. This report will present the serological status of Korean layers.

MATERIALS AND METHODS

1. Serum Samples

Among the serum samples received by CBNU-ADL from January to December in 2010, samples from various layer flocks were selected for the analysis of antibodies against 5 major viral disease of layers in Korea such as LPAI, ND, IB, EDS'76 and aMPV. Of the total samples, 1,319 samples were tested for AI 1,311 samples for ND and 1,145 samples were tested for IBV. The number of samples tested for EDS'76 and aMPV were 115 samples and 258 samples respectively, as samples collected from birds younger than 10 weeks were not submitted due to low quantity of samples.The time period was divided in to 7 phases by considering developmental characteristics and the effect of vaccine programs according to age- 0~1.3 wks (maternal antibody period), >1.3~3 wks (starting period), >3~10 wks (growing period), >10~22 wks (developing period), >22~40 wks (peak laying period), >40 ~60 wks (late laying period) and over 60 wks (post-molting period). The analysis of the first three weeks was excluded in cases of EDS'76 and aMPV as number of samples tested were too few. Further analysis was conducted on agents of interest considering the characteristics of each agent and vaccine program. In case of agents controlled by killed vaccine (LPAI and EDS'76), separate analysis of sero-positive flocks was conducted and individual titer against IB viruses were analyzed additionally due to the highly contagious nature of the agent; the positivity rate of aMPV in each flock was analyzed considering the abundance of the agent in Korea in 2010.

2. Serological Tests

Hemagglutination inhibition (HI) test and Enzyme linked immunosorbent Assay (ELISA) test were selected for serological testing using commercial diagnostic reagents or ELISA kits. The antibody titers for LPAI, ND, and EDS'76, which have hemagglutination activity, was measured by HI test as described in OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Sixth Edition, 2008). The antigens used for LPAI test was A/CK/Kor/01310/2011; La Sota strain was used for ND test and commercial EDS'76 diagnostic antigen (Daesung Microbiological Labs Co., Ltd, Gyeonggi) was used for EDS'76. Serologic surveys of IB, aMPV were conducted using IDEXX ELISA kit (Maine, USA) according to manufacturer's instructions.

3. Data Analysis

All serological data were analyzed using Excel 2000 (Microsoft Corporation, USA) and sero-prevalence, geometric mean titer, coefficient variant and SD value was calculated and the tendency by age was compared. In analysis of sero-prevalence, confidence interval was calculated only during analysis of overall age range due to the lack of data on population of for each week.

RESULTS

1. Sero-prevalence of 5 Major Viral Diseases of Layers.

For this study total 1,330 flocks were serologically analyzed against 5 major diseases such as AI, ND, IB, EDS'76 AND aMPV. According to Table 2, we confirmed that most layer flocks were immunized against Newcastle disease virus (NDV) and more than 75% of the flocks were immunized against other 4 agents in 2009. The total positive ratio were 73.8%, 99.7%, 97.1%, 78.2% and 78.0% against AI, ND, IB, EDS'76 AND aMPV respectively. The alterations by age of prevalence against 5 agents showed similar pattern. The prevalence were shown to be lowest at week 3 and increased

Table 1. The Number of flocks used in this study for serological tests against 5 major viral diseases of layers at each specific period of age

Diseases	The period of age (weeks)							Total
	0～1.3	>1.3～3	>3～10	>10～22	>22～40	>40～60	>60	
AI[a]	14	7	55	331	390	218	142	1,319
ND	14	5	69	228	384	266	154	1,311
IB	6	4	62	266	324	210	102	1,145
EDS'76	NT[b]	NT	NT	37	20	16	10	115
APV	NT	NT	NT	9	15	19	9	258

[a] Abbreviations used in this table as follow: Low pathogenic avian influenza (LPAI), Infectious bronchitis (IB), Avian metapneumoviral infection (aMPV) and Egg drop syndrome'76 (EDS'76).
[b] Not tested.

Table 2. Sero-prevalence of 5 major viral diseases in the samples submitted for regular or diagnosis in 2010

Diseases[a]	Total	The period of age (weeks)						
		0～1.3	>1.3～3	>3～10	>10～22	>22～40	>40～60	>60
AI (HI)	986 (73.8, 0.9)[b]	5/17 (29.4)[c]	8/36 (22.2)	10/55 (18.2)	212/357 (59.4)	341/390 (87.4)	229/284 (80.6)	177/191 (92.67)
ND (HI)	1,309 (99.7, 0.2)	20/20 (100)	9/10 (90)	87/90 (96.7)	357/357 (100)	384/384 (100)	266/266 (100)	192/192 (100)
IB (ELISA)	1,110 (97.1, 0.6)	11/11 (100)	15/32 (46.9)	49/62 (79.0)	318/320 (99.4)	338/339 (99.7)	226/226 (100)	161/161 (100)
EDS'76 (HI)	115 (78.2, 1.5)	NT[d]	NT	1/3 (33.3)	32/46 (69.6)	48/55 (87.3)	22/26 (84.6)	12/17 (70.59)
aMPV (ELISA)	202 (78.0, 4.6)	NT	NT	0/7 (0)	41/68 (60.3)	105/122 (86.1)	39/42 (92.9)	17/20 (85)

[a] Abbreviations used in this table as follow: Low pathogenic avian influenza (LPAI), Infectious bronchitis (IB), Avian metapneumoviral infection (aMPV), Egg drop syndrome'76 (EDS'76), Hemagglutination inhibition (HI) and Enzyme Linked Immunosorbent Assay (ELISA).
[b] Total number of sero-positive flocks (%, 95% coefficient interval).
[c] Number of flock seropositive/Number of flock tested (Positive ratio %).
[d] Not tested

until 22 weeks. The proportion was maintained with slight change in overall titer for the duration of the flock.

2. Observation of the Changes of Antibody Titers by Age

The serum antibody detected at 0～1.3 wks was regarded as being passively derived from breeder flocks. The lowest geometric mean was observed at >1.3～3 wks, which reflects the phenomenon of declining of maternal antibody in serum. The geometric means rebounded at >3～10 wks as a reflection of sero-conversion by vaccination or viral infection. There were 2 notable trends in change of antibodies observed at 22 wks, which is the period of egg laying and end stage of the vaccine program. One is that the geometric means developed at 22 wks were stabilized permanently for life; the other was that the geometric means continued to increase for life. The geometric means of AI, ND and EDS'76 were accorded with earlier type, stabilized for life, and those of IB and aMPV were accorded with the later, increased for life.

The coefficient variation (CV) was calculated to predict the

Table 3. Comparison of antibody titer against 5 major avian viral diseases at each specific period of age in the layer flocks submitted for diagnosis in 2010

Diseases[a,b]		The period of age (weeks)						
		0～1.3	>1.3～3	>3～10	>10～22	>22～40	>40～60	>60
AI	Total flock	0.85±0.34 (12.86)[c]	0.66± 0.20 (18.46)	0.68±0.23 (10.03)	2.78±0.70 (28.31)	4.4±0.99 (27.05)	4.25±0.86 (26.45)	4.94±1.03 (26.08)
	Positive flock	2.90±1.17 (43.72)	2.98±0.92 (83.08)	3.77±1.25 (55.16)	4.58±1.16 (46.57)	5.00±1.12 (30.76)	5.22±1.06 (32.52)	5.33±1.11 (28.14)
ND	Total flock	6.35±1.01 (16.27)	4.28±25.52 (0.93)	5.29±1.24 (39.53)	7.31±1.16 (17.04)	7.50±1.03 (14.48)	7.38±1.00 (14.07)	7.76±1.07 (13.71)
IB	Total flock	5,198.17±996.23 (36.22)	1,525.59±759.30 (87.20)	3,250.16±1,375.21 (66.81)	5,129.90±2,041.23 (42.69)	6,501.66±2,318.08 (35.83)	6,472.54±2,382.50 (35.71)	7,128.43±2,463.74 (33.85)
EDS' 76	Total flock	NT[d]	NT	NT	4.09±2.89 (40.97)	4.24±1.09 (41.42)	4.00±1.27 (34.71)	4.57±0.78 (27.45)
	Positive flock	NT	NT	NT	5.88±4.15 (58.89)	4.85±1.24 (47.46)	4.73±1.33 (41.03)	6.48±1.10 (38.88)
aMPV	Total flock	NT	NT	42.08±104.62 (125.29)	2,105.74±1,812.66 (83.02)	4,291.78±3,448.63 (77.00)	5,138.87±3,757.23 (69.42)	6,670.03±5,568.22 (88.01)

[a] Abbreviations used in this table as follow: Low pathogenic avian influenza (LPAI), Infectious bronchitis (IB), Avian metapneumoviral infection (aMPV), Egg drop syndrome'76 (EDS'76).
[b] Mean antibody titer was calculated from total flocks for ND, IB and aMPV, and from both total and positive flocks for AI and EDS'76.
[c] Geometric mean titer±Standard Deviation (Coefficient of variation).
[d] Not tested.

degree of dispersion. Values less than 30% were regarded as reflection of low dispersion. CV value was lowest in ND compared to any other agents analyzed in this study. Except at >3～10 wks, the coefficient variation for ND was lower than 20%.

3. The ELISA Titers of Specific Antibodies of Individual Birds against IB Virus

In case of IBV, the detection of infection titer indicates the infection of the flock regardless of geometric means. Thus, to estimate the scale of field infection, individual titer should be considered when analyzing IBV antibody titers. Fig. 1 presents the ELISA titers of individual birds by age. Titers higher than 12,000 was regarded as reflection of field infection in flock. Infection titer emerged as early as 10 weeks and the titer permanently remained for life. Considering that the upper threshold of vaccine titer did not exceed 6,000 in previous report (Lee et al., 2010), initial infection by IB field strain may have started as early as 10 weeks and infection may be

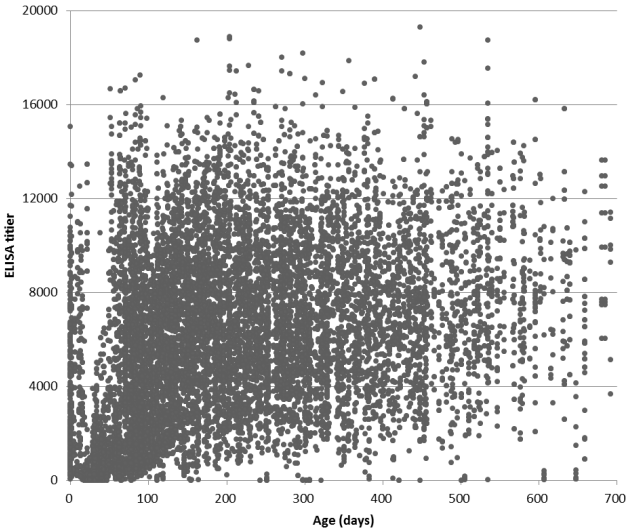

Fig. 1. The comparison of individual antibody titer against infectious bronchitis virus from the layer flocks submitted for diagnosis in 2010.

repeated by various strains throughout the whole lifetime.

4. Sero-Prevalence of aMPV in Flocks at 2010

Fig. 2 represents the prevalence of seropositive birds in each flock according to respective age groups. There were flocks which were shown to be aMPV negative before reaching 40 weeks. However, flocks tested after 40 weeks included at least one bird which turned out to be sero positive for aMPV. 100% sero positive flocks emerged as early as10 weeks and the positivity was maintained for life.

DISCUSSION

Serum antibody titers have a tendency to change depending on flock condition and conclusions for diagnosis are made based on the changes of antibody titers (Butcher, 2002). Increase of titers may indicate recent exposure to the suspected agent. In forms of serum antibody development, vaccination and exposure to field agents is the most common route of antigen exposure for modern farms, with the latter still remaining as a problem. Thus, consideration of vaccination strategy and possibility of field infection are critical for proper interpretation of data. Based on these factors, we can categorize diseases into two types; the diseases which can be controlled effectively by implementing frequent vaccination programs and the diseases which could not be controlled effectively due to low efficiency or even absence of efficient vaccination programs, allowing interference of field infections in developing serum antibodies.

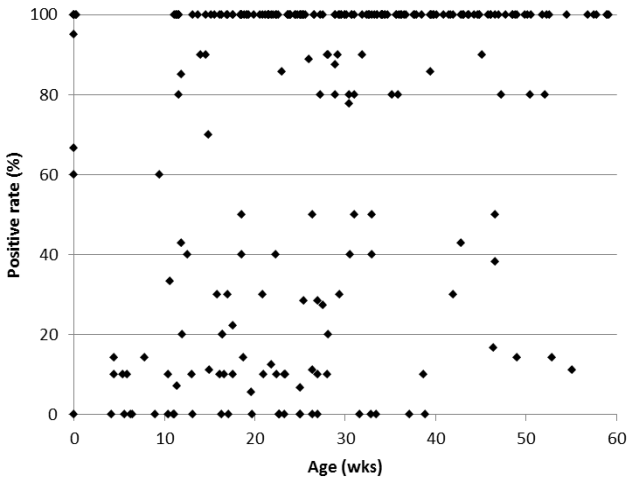

Fig. 2. The comparison of positive rates against avian metapneumovirus from the layer flocks submitted for diagnosis in 2010.

The number of samples submitted for serological analysis was optimized based on the fact that the total number of layer farms in Korea in September 2010 was 1,538 (National Statistical Office, Korea) and the formula $n = Z^2PQ/L^2$ (Perminand Hansen, 1998), wheren=sample size, P=expected prevalence, Q=1−P, L=required precision, the optimized sample size for analysis of overall sero-prevalence 432±4 in 95% confidence level. Table 1 shows the number of farms used in this study.

LPAI and ND appeared to belong to the previous type, effectively controlled type, of the diseases. In case of LPAI, the initial rise of titer was consistent with the period of first vaccination (3～10 wks), which was conducted following the popular conventional LPAI vaccine program in Korea (Lee et al., 2010). However, it is not clear if humoral immunity organized at 0～1.3 wks can effectively protect flocks against LPAIV. CV was lower than 30% in case of LPAI and 20% for ND which means that the antibody titer against these two diseases was formed uniformly. In case of ND, the tendency is more prominent as vaccination is conducted more intensively than any other disease. The amount of antibody detected at 0～1.3 wks (Table 3) confirmed that maternal antibody was transferred to enough quantity to protect young chicks from systemic infection of NDV. The temporal correlation between vaccination and increase of antibody titer supports the assumption that the serum antibodies developed against each respective disease were mostly due to vaccination. Moreover, considering that the outbreak of these two diseases was extremely rare at 2010, the protective effect of vaccination was reflected in this study as highly-stabilized GMT value of antibody titers.

IB and aMPV infections can fall into the second category of diseases as previously described as the vaccine strategy against IB are known to be incomplete because of many serotypes of IB viruses and vaccine programs and vaccines against aMPV were non-existent at 2010. In case of IB, the infection titer (≥12,000) started to show up around 10 weeks and eventually lasted for life (Fig. 1) despite intensive IB vaccination. It is also unclear if maternal antibodies formed at 0～1.3wks are enough to provide protection against IBV.

In previous studies (Lee et al., 2010), it was proved that the ELISA antibody titer of IB developed by vaccination could not exceed 7,000, so infection titer exceeding 12,000 means exposure to field viruses. According to the data, the field infection started as early as 10 weeks and the agent was re-introduced continuously for life. Flocks with 100% positive bird rate against IB also found around 10 weeks such as 99.4% at 10~22 weeks old of age. In case of aMPV, the result was consistent with previous studies which reported high prevalence of aMPV in layers and broilers (Park et al., 2011). In this study, the positive ratio were observed at high levels (>60%) and every flock over the age of 40 weeks had developed antibody against aMPV (Fig. 2). Overall CV of aMPV was higher compared to other diseases in each age group while there was no apparent pattern by change. However, it was difficult to interpret the change of titer due to wide variation among data and scarcity of information on aMPV. These results demonstrate the abundance of aMPV infection but detailed analysis is still required to verify clinical significance.

In case of EDS'76, it was difficult to determine the disease into specific categories due to the scarcity of samples. However, by comparing with 2009 (Lee et al., 2010), decrease in positivity appears to correlate with the tendency of reduced frequency of vaccination against EDS'76.

In addition to the major factors previously mentioned, we could not rule out the effect of two important events which occurred at 2010. One is the official field test of aMPV vaccine which was developed by local company (Green cross Veterinary products, Yongin Korea) and applied to layers and breeders throughout country. The impact of the field test seems to be reflected directly in our tests in form of decreased CV value, which suggests that the titers for aMPV became more stable than those of 2009 (Lee et al., 2010). It is not certain whether this event had influenced GMT value due to the scarcity of serologic data of aMPV. The other event is the outbreak of Highly Pathogenic Avian Influenza (HPAI) in breeder flock in late 2010. The outbreak of HPAI severely limited supply of new flocks which led to delay of culling in many old flocks. The outbreak was also reflected in this study as many flocks which were tested were over 60 weeks. It could be also observed that GMT value of LPAI did not drop after 40 weeks in this study, implying the possibility of increase in the frequency of revaccination against LPAI after sixty weeks.

In conclusion, this study displayed not only general trends of major viral disease in layers, but put occasional events in account which occurred at 2010. Considering the trend of development of preventive measures against major viral diseases and the evolution of agents of interest, implementation of regular annual serologic analysis are critical for accurate interpretation.

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