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# Flock-level Seroprevalence of and Risk Factors for Infectious Bronchitis Virus in Korean Laying-hen Flocks

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**Abstract :** Although there is circumstantial evidence that infectious bronchitis (IB) in the Korean layer industry has contributed to severe economic losses, the seroprevalence against IB virus (IBV) and risk factors associated with seropositivity are not well known. During May to October 2007, 820 blood samples were randomly collected from 41 laying hen flocks (20 birds in each flock) with ≥ 3,000 birds of 18 week of age or older in three provinces of Korea. The samples size was determined considering a flock-size range of 3,000–65,000 birds, an expected bird-level seroprevalence of ≥ 15%, and a 95% level of confidence. Serum samples were examined using a hemagglutination inhibition test for antibodies to IBV. The overall apparent flock-level seroprevalence was 46.3% (95% CI, 31.1–66.6) with no statistically significant differences among provinces ($X = 1.205$, $p > 0.05$). There were 19 positive flocks with one to eight seropositive birds, and 11 of these had one or two seropositive birds. None of the measured parameters were significantly associated with seropositivity against IBV in a subsequent multivariable logistic regression analysis. A longitudinal risk factor studies considering management and vaccination characteristics possibly associated with the IBV flock prevalence would be beneficial.

***Key words*** **:** infectious bronchitis, layer, risk factor, seroprevalence.

## Introduction

Infectious bronchitis (IB) is a primarily respiratory disease of chickens, and is prevalent within the poultry industry in many parts of the world (4). Economic losses due to IB virus (IBV) infection are often complicated depending on the IBV strains and secondary infections (9). More importantly, it has been well documented that IB is still problematic even in areas where vaccines have been implemented (4,7,15), and IBV strains that were circulated are often found to be different from vaccinal strains (1,5,16). The knowledge that what percentages of flocks in a country are actually infected and which risk factors are significantly associated with IBV infection is critically important for developing of a national control program.

In Korea, the first report of IB was from a 1986 study of breeding laying hens with a major clinical history of reduced egg production in Chungnam province (17). Historically, both respiratory and nephritic forms of IB have occurred in Korean laying flocks for over two decades, with severe economic losses (17,18). Despite the use of both live attenuated and inactivated oil-emulsion vaccines, the problem still exists. This might be caused in part by the many antigenic types of the causative agent and the lack of IB-specific clinical signs recognized by practitioners and farm owners. In addition, vaccination schedules on farms vary greatly, depending on the veterinarian. IB in Korea is considered to be of lesser importance because of the relatively low mortality and small economic losses compared to other infectious diseases such as Newcastle disease, which has long been targeted by the government for eradication. Although IB is assumed to be the second most prevalent disease in Korean layer industry, estimates of IBV seroprevalence and risk factors related to seropositivity at the farm level is not available to date. In response to growing animal health and economic concerns, an epidemiological project was started in May 2003; its primary aim is to develop effective monitoring procedures for IB. Here, the author present a preliminary study estimating the seroprevalence of IB in randomly sampled laying hen farms and the association of seropositivity with some selected risk factors.

## Materials and Methods

### Design of sample collection

A cross-sectional study was conducted from May through to October of 2007 in three provinces of central and eastern Korea: Gangwon, Chungpook, and Chungnam. According to the statistics of the Ministry of Food, Agriculture, Forestry, and Fisheries (MFAFF), as of December 2006, approximately 405 (20.9%) of 1,934 laying hen farms were operating, representing 25% of all laying hens (approximately 57,000,000). All farms that had 3,000 or more laying hens of

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18 week of age or older were eligible to participate in the study because this size is the basis for the estimation of quarterly statistics by the MFAFF, and the number of individual farms with < 3,000 birds is not accurately known. Within each of the three provinces, about 10% of all farms (11 of 99 farms in Gangwon, 10 of 100 in Chungpook, and 20 of 206 in Chungnam), with one flock for each farm, were recruited randomly using a random number table from records that were maintained by local veterinarian practices. This sample size provided a 95% confidence level for an expected flock-level prevalence of 50.0±15%. All owners voluntarily agreed to allow project personnel to visit their flocks, determine the IB infection status of the farm, and assess specific risk factors. Blood samples from 20 birds in each flock were collected for serological testing, for a total of 820 blood samples. This within-flock minimum sample size of birds was determined to achieve 95% confidence at an expected bird-level seroprevalence of ≥ 15% and a flock-size range of 3,000–65,000 birds (3).

### Hemagglutination inhibition test

Each blood sample was collected via venipuncture of the wing vein. Upon arrival at the laboratory, blood samples were centrifuged for 10 min for serum separation and stored at −20°C until analysis. Four HAU (hemagglutination unit) of antigen (Massachusetts serotype; DaeSung Microbiological Labs, Korea) was added to serial twofold dilutions of kaolin-treated serum. Washed chicken erythrocytes at a concentration of 1% were added to the diluted antigen and serum mixture after incubation for 30 min at 4°C; the test was read after incubation for 40 min at 4°C (2,11,12). The titer was expressed as a logarithm (base 2) of the reciprocal of the highest dilution of serum that gave 100% inhibition of hemagglutination (HA). An IBV titer ≥ 10 was considered positive with the consultation of diagnostic experts. At the time of sampling, questionnaire-based information was collected with the help of trained veterinarians to identify risk factors that may be associated with IB seropositivity. These included managerial characteristics of the farms, environmental conditions, breeds of hens, disinfection practices, ventilation system, manure disposal methods, control of vehicle movements, and rodent and fly control practices.

### Statistical analysis

Data gathered from the survey were entered into a Microsoft Excel (Redmond, WA) spreadsheet. Taking into account the clustering of positive birds within each farm, a generalized linear mixed-effect model was used that included a random effect of farm. The analysis was performed using the glimmix macro in Statistical Analysis System (SAS Institute, Inc., Cary, NC), with seropositivity as an outcome variable. A total of 17 independent variables (4 continuous and 13 categorical) were analyzed using univariate and multivariate analyses to produce odds ratio and corresponding confidence interval (CI) for the associations between the outcome and risk factors. All continuous independent variables were left unaltered into the model, but dummy variables were created for each categorical variable for inclusion in the multivariable analysis (8). Variables with $p \leq 0.25$ were chosen for further evaluation using multivariable procedures. Two-way interactions were not considered to simplify the model interpretation. The level of significance was set at $p < 0.05$. In this study, seropositive flocks were defined as those having at least one sample that was positive for IBV, and the apparent seroprevalence with 95% CI was calculated without correcting for the sensitivity and specificity of the HA inhibition test. The provincial differences for flock seroprevalence were compared using the chi-square test ($p < 0.05$).

## Results

### Flock-level seroprevalence

Of the 41 flocks surveyed, 19 (46.3%; 95% CI, 31.1–66.6%) were serologically positive for IBV; 6 (54.5%; 95% CI, 25.1–84.0%) in Gangwon, 4 (40%; 95% CI, 9.6–70.4%) in Chungpook, and 9 (45%; 95% CI, 23.2–66.8%) in Chungnam. There was no statistical difference in flock seroprevalence among the provinces ($X = 1.205$, $p > 0.05$). The 19 positive flocks had one to eight seropositive birds; of these, 11 had one or two seropositive birds (Table 1).

### Risk factors

Of the 17 risk factors examined, five were initially associated with seropositivity at $p \leq 0.25$. These included longer duration of farm operation, multiple ages on a farm, occa-

**Table 1.** Seroprevalence of chicken infectious bronchitis virus in layers, by surveyed province, in Korea (May-October 2007)

| | Province | | | |
|---|---|---|---|---|
| | GA | CP | CN | Total |
| No. flocks tested | 11 | 10 | 20 | 41 |
| No. positive flocks | 6 | 4 | 9 | 19 |
| Apparent prevalence (%)[a] | 54.5 | 40.0 | 45.0 | 46.3 |
| (95% CI) | 25.1-84.0 | 9.6-70.4 | 23.2-66.8 | 31.1-66.6 |
| Distribution of positive flocks | | | | |
| 0 | 5 | 6 | 11 | 22 |
| 1 – 2 | 4 | 2 | 5 | 11 |
| 3 – 5 | 1 | 2 | 4 | 7 |
| > 5 | 1 | 0 | 0 | 1 |
| Total | 11 | 10 | 20 | 41 |

CI=confidence interval, GA=Gangwon, CP=Chungpook, CN=Chungnam.
[a]Chi-square = 1.205, p > 0.05.

**Table 2.** Odds ratios (ORs) and 95% confidence intervals (CIs) of explanatory variables associated with seropositivity for chicken infectious bronchitis (IB) virus in layers in Korea, May to October, 2007

| Risk factors | OR | 95% CI | p value |
|---|---|---|---|
| Farm operation (years) | | | |
| < 10 | 1.0 | - | - |
| ≥ 10 | 1.1 | 0.9, 3.0 | 0.132 |
| Presence of multi-age flocks | | | |
| No | 1.0 | - | - |
| Yes | 1.9 | 1.1, 3.2 | 0.045 |
| Disinfection practice | | | |
| Always | 1.0 | - | - |
| Occasional | 1.3 | 0.9, 2.7 | 0.156 |
| Ventilation system | | | |
| Forced | 1.0 | - | - |
| Natural | 1.2 | 0.8, 1.6 | 0.207 |
| Flock size | | | |
| 3,000 – 15,000 | 1.0 | - | - |
| > 15,000 | 1.2 | 0.5, 1.3 | 0.105 |

sional disinfection procedure, natural ventilation system, and larger flock size (Table 2). No variables were significantly associated with IB seropositivity in the final logistic model.

## Discussion

Approximately half of the flocks examined were infected with IBV, indicating that IB infection is widely distributed in the project area and is responsible for a large proportion of the respiratory disease on the study farms. This high seroprevalence of Korean laying hen flocks in together with a highly contagious nature of the disease suggest that the control program will only be effective if most farms participate. From the literature, field studies using Massachusetts-type antigen have revealed flock prevalences for IBV infection of 56.5% of 30 backyard chicken flocks in Mexico (7), 100% of 16 layer flocks (66.6% of 9 broiler flocks) in Pakistan (1), 50% of 236 broiler flocks in the United Kingdom (15) and 92.5% of 40 fancy breed poultry flocks in Switzerland (19), although serological test in this study is different from the latter two studies (polymerase chain reaction and enzyme-linked immunosorbent assay, respectively). At this time, it is not clear whether the prevalence of 46.3% found in this study truly reflect the changes in prevalence in the study population owing to the increased awareness of the disease, but they do deserve consideration in a future study. On the other hand considering the endemic disease in Korean layer farms, the results may be an underestimate. In this case, there may be several explanations for this. The most probable is that only one serotype (Massachusetts) was used as an antigen, although this serotype gives more cross-reactions than do other serotypes (2,13). This is supported by the results of an epidemiological study in which the authors reported that at least four genetically and serologically different variant serotypes of IBV are prevalent in Korea (18). Genetic diversity within IBV has also been documented worldwide (14). Another explanation may be that the study was performed over a limited time period, and project personnel only visited each farm once. This strategy may not have provided sufficient opportunity to detect infected birds, although birds of all ages may be susceptible to IBV (10). In addition, the number of individual birds to be sampled in each flock was calculated statistically, but may not have been the best sample size to reveal seroprevalence because of a lack of sufficient information for the calculation. When the study was in the planning process, there was no reasonable estimate of the seroprevalence of antibodies to IBV in the project area. The minimum number of flocks to be sampled per province could be determined by considering the number of flocks present in each province (range: 99–206), an expected flock-level seroprevalence of 50% (maximum sample size), and a desired accuracy of 10% for a 95% level of confidence (3). This resulted in 49 flocks to be sampled in Gangwon and Chungpook each and 66 flocks in Chungnam. Accordingly, only 25% of the total of 164 calculated flocks was examined in this study, mainly because of financial reasons. Lastly, the diagnostic threshold (HI titer ≥ 10) used to classify seropositivity might have been too strict, yielding a large number of false-negative results. This criterion was rather arbitrary, but was based on the experiences of diagnostic experts after considering vaccination practices in the field. In an experimental study with broilers, IBV titers in HI test ≥ 7 were suggested for IB serotyping (6). Gutierrez-Ruiz et al (7) used titers ≥ 4 for serological survey in an area where minimal vaccination is practiced. Any of these factors and study limitations may have affected the apparent prevalence detected.

The small sampling number, as mentioned above, may have contributed to the failure to demonstrate the significance of any risk factors in the model. Despite the wide distribution of IBV in many countries, to the author's knowledge, there is very limited evidence in the literature describing the risk factors for IBV in layer flocks. Further large-scale studies are needed to re-examine this issue taking into account the aforementioned factors.

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# 국내 산란계에서 닭 전염성기관지염의 계군 수준 유병율과 위험요인

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**요 약 :** 국내 산란계에서 전염성기관지염 (IB)에 의한 경제적 피해가 심각하지만 IB 바이러스 (IBV)에 대한 혈청학적 유병율과 위험요인에 대한 연구는 잘 알려져 있지 않은 실정이다. 2007년 5월부터 10월까지 강원, 충북 및 충남 지역의 3,000수 이상을 사육하는 41개 산란 계군 중 18주령 이상을 대상으로 총 820수 (계군 당 20수)에서 혈액시료를 채취하였다. 이러한 표본크기는 평균 계군 크기 3,000-65,000수, 최소 기대 유병율 15%, 95% 신뢰수준을 고려하여 계산하였다. 혈액시료는 혈구응집억제검사를 사용하여 IBV 항체역가를 측정하였다. 41개 계군 중 19개 계군이 양성으로 확인되어 계군 수준의 유병율은 46.3% (95% CI, 31.1–66.6)로 지역별로 유의한 차이는 없었다 ($X = 1.205$, $P > 0.05$). 전체적으로 계군 당 1-8수가 감염 역가를 보였으나 양성 계군 중 11개 계군에서는 1-2수만이 감염역가를 보였다. 로지스틱모형을 이용한 위험요인 분석에서 IBV 혈청양성과 연관된 유의한 변수는 없는 것으로 나타나 향후 광범위한 산란 계군을 대상으로 사양관리와 백신접종 등을 고려한 종주연구가 필요할 것으로 사료된다.

**주요어 :** 닭 전염성기관지염, 산란계, 위험요인, 유병율.