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Risk factors for infectious bronchitis virus infection in laying flocks in three provinces of Korea: preliminary results

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Abstract : To analyze and identify selected risk factors for infectious bronchitis virus (IBV) infection in the growing and laying period of laying-hen flocks, a longitudinal field study was conducted with 27 commercial flocks reared in three provinces of Korea during the period from May 2003 to April 2004. Using monitored data for IBV infection status among study flocks we computed the multivariate odds ratios (ORs) and their corresponding confidence intervals (CIs), and population attributable risks (PARs). Multivariate logistic regression showed significant risk increments for: continuous entry of chick (OR=1.9, 95% CI, 0.7-69.1) and operation years of the layer house greater than or equal to 5 years (OR=3.2, 95%CI, 1.6-389.9). No significant interaction was found between variables. The PAR suggested that continuous entry of chick (PAR=32%) and ≥5 years of house operation (PAR=84%) had the highest impacts on IB presence in laying-hen flocks under study. Of the two significant factors, however, operation year of the layer house lacks an easy applicability in preventing IB control strategies, and the possibility of confounder cannot be ruled out.

Key words: longitudinal study, chicken, risk factors, infectious bronchitis

Introduction

In Korea, since the first case of infectious bronchitis (IB) was reported in laying breeders in 1986 [16], sporadic outbreaks of infectious bronchitis virus (IBV) infection have occurred in laying flocks throughout the country, and the incidence is the second the most prevalent in the layer industry. From the literature, both respiratory disease and nephritis form of IBV infection have been occurring on Korean laying flocks for over two decades [10, 16], resulting in severe economic losses. Recently, results from a retrospective study indicated that IB was present before 1986 [14].

Currently, although both live attenuated vaccines and inactivated oil-emulsion vaccines are being applied to prevent and control the incidence of the disease among laying and broiler flocks, the disease incidence has not decreased. This might be in part due to not only many antigenic types of the causative agent, but also lack of sufficient field information available to recognize IBV infection for practitioners and farm owners. Of importance is that IB has been considered as being less important for practitioners and farm owners because of the relatively low mortality and less economic loss compare to other diseases such as Newcastle disease, which has long been targeted by the government for eradication. In some cases, poor weight gain and feed efficiency and reduction of egg production caused by IB were not significant to be noticeable by farm owners.

In response to growing animal health and economic concerns and a continuing high incidence of IBV infection in Korea, a comprehensive epidemiologic project (infectious bronchitis project, IBP) was carried out. The project began in May 2003 and had the following 2 objectives: to elucidate the major factors

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for IBV infection in layer-hen flocks and to develop effective monitoring procedures to reduce economic losses caused by this disease. The purpose of the study reported herein was to analyze data for the first 12 months of the project for a subset of 27 flocks and to identify some selected risk factors associated with IB among layer-hen chickens.

Materials and Methods

Study population and sampling

The IBP involved only those flocks whose owners had volunteered to allow personnel to visit their flocks, determine IB infection status of the flock and assess risk factors under consideration in the IBP. Briefly, the target population of this study was composed of commercial laying-hen flocks reared in Gyunggi, Chungpook, and Kangwon province between May 2003 and April 2004. Flocks in areas (sampling frame) represent 25% of all Korean layer-chicken owners and 35% of layers. Twenty seven flocks of the original 31 flocks which were monitored for IBV infection were included in the analysis. For serologic test, blood samples from 10 hens were selected from each flock at monthly-basis, based on a 95% confidence in selecting at least one seropositive hen, assumed 30-35% of assumed level of prevalence [4]. For practical purposes, flocks in different houses on a single farm and flocks housed in the same house but at different times were considered separate flocks.

Serology

Each blood sample was collected in a 5 ml tube (Vacutainer; BD, USA). After approximately 12 h at room temperature, blood samples were centrifuged at 1500 g for 10 min for serum separation and stored at -20°C until analysis. A flock was considered positive if there is at least one layer hen with HI titer (log_2) ≥ 11 for IBV. The hemagglutination inhibition (HI) test was done by adding 4 hemagglutination (HA) units of HA antigen (DaeSung Microbiological Labs., Korea) to serial two fold dilutions of each kaolin-treated test serum [1, 11, 12]. RBCs (1%) were added to HA antigen-serum mixture after incubation 30-min at 4°C, and the test was read after a 40-min incubation at 4°C. HI titers were expressed log_2 of the reciprocal of the highest dilution of test serum causing inhibition of HA.

Data collection and analysis

(1) Variable description

Data on IB infection status from each study flock was recorded on standard forms by the IBP personnel, which was then entered into a data base. The following information on 8 independent, flock-level variables was collected: ① Strain of layer (1=Hyline, 2=Roman). ② The flock size as expressed by the number of chicks housed (1: 1-15000, 2: >15000). These two levels of size classification were chosen to avoid small groups. ③ Age as expressed by phase of laying cycle [1: ≤17 weeks (before laying), 2: 18-34 weeks (early phase), 3: 35-56 weeks (middle phase), 4: > 56 weeks (late phase)]. ④ Method of chicken entry on the farm (1=all-in-all-out, 2=continuous). ⑤ Expected season with highest egg production (1=spring or summer, 2=fall or winter). ⑥ Appropriate use/installment of ventilation system (1=yes, 2=no). ⑦ Operation of the layer house in years (1: < 5, 2: ≥ 5). ⑧ Possibility of cold stress in the house (1=no, 2=yes).

(2) Evaluation of risk factors and logistic regression analysis

Unconditional logistic regression was performed, with the flock-level IB infection status (yes vs no) as outcome variable. All the independent variables were initially analyzed by use of a univariable regression analysis to develop crude odds ratios (OR) and the corresponding confidence intervals (CIs) for the association between outcome and risk factors. Variables with a p-value ≤ 0.25 were chosen and presented for further evaluation using multivariable procedures. A forward- and backward selection method in which the likelihood-ratio test was used to assess the significance of adding or subtracting one variable at a time from the model to obtain adjusted OR estimates for the other factors in the model. The goodness-of-fit of the model was determined using the Hosmer-Lemeshow statistic [8]. The statistical analyses were performed in the Statistical Analysis System (SAS, Cary, USA) [17]. A value of $P<0.1$ was considered statistically significant.

(3) Population attributable risk (PAR)

The PAR was obtained by an approach based on unconditional logistic regression [3]. The PAR was determined using the following formula: $PAR = 1-\sum_j (P_j / R_j)$, where R_j estimated relative risk for each stratum (j) of each variable in the final model, and P_j the proportion of all flocks with IBV positive within stratum j. By combining adjusted OR estimates derived through logistic regression and the observed prevalence of the risk factors under study in the cases (positive for both IBV), this approach yields adjusted PAR estimates [20]. Estimates of PAR range between 0 and 1 (or 0% and 100%), but when the factor under study appears protective, a negative PAR is derived. PARs were estimated for each separate risk factor and for combinations of risk factors. The summary PAR for variables with p-value of ≥ 0.25 in the final model was determined using the above formula for PAR.

Results

Thirty of the 31 original study flocks which were able to be periodically monitored for IBV infection were seropositive at least once during the observation period. At the time of analysis (May 2004), the data included a total of 27 flocks, of which 12 were infected with IBV and 15 were not.

All flocks were housed in high-rise facilities. Initial evaluation of contingency tables for each of 9 variables cross-classified with the outcome suggested that 2 variables, method of chicken entry to farms and house operation were strongly associated with the IBV infection (Table 1). A flock operation with greater than 5 years operation was 12.6 (95% CI, 1.3-123.6) times as likely to be infected for IBV as was a flock with less than 5 years operation. No two-way interaction terms were identified between the variable, operation and other covariates. In the final model, continuous entry of chick (OR = 1.9, 95% CI, 0.7-69.1) and greater

Table 1. The distribution of the independent risk factors with flock-level IB infection status in a field study of IBV infection in 27 layer flocks (Korea, 2003-2004)

Risk factor	IBV positive		IBV negative		Odds ratio (95% CI)	p-value
	No.	%	No.	%		
Age						
≤17 weeks	4	33.3	7	46.7	Reference	
18-34 weeks	4	33.3	2	13.3	3.5 (0.4 - 28.5)	0.2416
35-56 weeks	3	25.0	5	33.3	1.1 (0.2 - 6.9)	0.9596
> 56 weeks	1	8.4	1	6.7	1.8 (0.1 - 36.3)	0.7175
Strain of layer						
Hyline	7	58.3	7	46.7	Reference	
Roman	5	41.7	8	53.3	0.6 (0.1 - 2.9)	0.5476
Flock size						
1-15,000	3	25.0	3	20.0	Reference	
> 15,000	9	75.0	12	80.0	1.3 (0.1 - 4.6)	0.7565
Method of chicken entry						
All-in-all-out	6	50.0	4	26.7	Reference	
Continuous	6	50.0	11	73.3	2.8 (0.6 - 13.8)	0.2180
Peak season						
Summer	8	66.7	9	60.0	Reference	
Winter	4	33.3	6	40.0	1.3 (0.2 - 9.0)	0.8249
Ventilation system						
Appropriate	8	66.7	6	40.0	Reference	
Inappropriate	4	33.3	9	60.0	1.3 (0.1 - 1.6)	0.1740
Operation years of the layer house						
< 5	1	8.3	8	53.3	Reference	
≥5	11	91.7	7	46.7	12.6 (1.3-123.6)	0.0299
Cold stress						
No	3	25.0	4	26.7	Reference	
Yes	9	75.0	11	73.3	0.9 (0.2 - 5.2)	0.9220

Table 2. The results of the final logistic regression model with flock-level IB infection status in a field study of IBV infection in 27 layer flocks (Korea, 2003-2004)

Risk factor	Parameter estimate	Odds ratio (OR)	95% CI of OR	p-value
Continuous entry of chick	1.91	6.7	0.7 – 69.1	0.10
≥5 years of layer house operation	3.21	24.7	1.6 – 389.9	0.02

than equal to 5 year-old house operation (OR = 3.2, 95%CI, 1.6-389.9) were significant (Table 2). This model fit the observed data well by -2log likelihood ratio (Chi-square = 6.76, df = 1, $P < 0.05$).

The estimates of PAR for individual variables and a summary PAR for all variables are shown in Table 3. Based on the results from Table 2, the following logistic model was constructed: P = 1/[1+exp -(4.61-2.53×operation in years)], where, P represents the probability of being IBV infection. Using this formula, the estimated probabilities of the farm being IBV infected by operation years are shown in Table 4. A farm with less than one year of operation has 11% probability of IB infection and this probability increases steeply, reaching 99.9% in a farm with more than 5 years of operation.

Discussion

High IB prevalence of 96.8% suggests respiratory type infection is widely distributed during all seasons and IBV may be responsible for a large proportion of the respiratory disease in the study flocks. It has been reported that IBV is endemic at the majority of poultry sites, with an incidence approaching 80-100% [7, 9, 19].

In the beginning of the analysis, the authors considered three variables (method of chicken entry, ventilation system, and cold stress) as potential risk factors for IBV infection, but none were statistically significant. This result could be attributed to not only lack of sufficient data sets, but also to wide distribution of risk factors in the study population, resulting in masking of true risk with false relationship. At this time of analysis, this assumption could not be validated and need to be studied in the future study.

Table 3. Population attributable risk (PAR) for significant factors in the final model which were associated with IB positive

Variable for risk factor	PAR
Continuous entry of chick	0.32
≥5 years of layer house operation	0.84
Two variables combined	0.89

Table 4. Estimated probability of being IB infection in a farm by operation year

Operation (year)	Estimated probability of IB infection (%)
1	11.1
2	61.1
3	95.2
4	99.6
≥5	99.9

The finding that estimated 32% or 84% cases of all farms with IBV positive could be removed by eliminating or modifying the effects of continuous entry of chick or being in a house of ≥5 years, respectively, is of epidemiological importance. From this analysis, it was estimated that 89% of all case flocks studied could be removed by designing alternatives that reduce the effects of these 2 risk factors to their baseline levels. However, this estimate of PAR must be interpreted with caution, given that the identical risk factors may be acting through pathways that involve other factors not addressed in this study. This is likely to bias PAR upwards. The PAR for individual risk factors do not add up to the summary PAR, indicating that these factors are not mutually exclusive [3].

To the at least author's knowledge, none of the countries which have an intensive poultry industry under flock management with all-in/all-out operations, cleaning and disinfection, are free from IBV. The only aggressive means of controlling IB is vaccination, but exclusion of IBV has not been achieved through such measures, even in vaccinated flocks of all ages [6, 15, 21]. Judging from the current epidemiological study, vaccination programs and procedures differ from one province to another or even from one farm to another within the same province, depending on local conditions or veterinarian's preference. Actually, IB in Korea is thought to be endemic at the majority of poultry farms despite the wide use of IB vaccines. Possible explanations for high prevalence of IB in Korean laying-flock are continual cross-infection within infected or vaccinated flocks, continuous excretion of the virus at levels usually below the detection levels of tests [2], long-term duration of IBV infections due to incomplete removal of the virus from the source farm and re-activation after latency [5]. Of the other factors, inappropriate storage or transportation of vaccines, poor management practice of farm or its environment such as insect control and ventilation may also be controlled. In particular, a multitude of IBV serotypes exist [18] and this has become the major problem in diagnosis and control of IB in the country, yielding vaccine failure due to not protecting against the prevalent antigenic type. Many other factors involved in the IB infection, which are based on author's experiences and expertise's opinions are: variant serotypes of circulating Korean IBV field isolates [18], wide-spread use of live attenuated vaccine with different vaccination schedule by practitioners [13], co-circulation of vaccinal-type virus and wild strains and risk of ignoring or unknowing a subclinical IBV infection.

One important finding in this study is that the variable, operation year of the layer house, showed a significant association with increased risk of IB infection. The authors at the time of preparing this paper could not determine the possibility of confounding factor due to lack of sufficient data; but in this study those farms with operation years of five or higher, the facilities and management practices of the house were at higher risk for other diseases. Further studies are, therefore, very much needed to determine whether this variable may play an important risk factor or just confounder, even after controlling for unknown factors.

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