An Epidemiological Investigation of Avian Reovirus Among Commercial Broiler Chicken Flocks in Ontario

by

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ABSTRACT

AN EPIDEMIOLOGICAL INVESTIGATION OF AVIAN REOVIRUS AMONG COMMERCIAL BROILER CHICKEN FLOCKS IN ONTARIO

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This thesis is an investigation of prevalence, geographical distribution, and seasonal variation, and risk factors of avian reovirus (ARV) among commercial broiler chicken flocks in Ontario. Avian reovirus prevalence in the study population was 90.5% (95% confidence interval: 86.8 to 94.3%). Using univariable logistic regression models, statistically significant associations were identified between ARV presence and geographic district ($p = 0.012$). There was no association between flock mortality and flock ELISA mean titre or PCR status. In the multivariable linear regression model and multi-level logistic regression model with flock as a random effect, co-infection with chicken anemia virus was associated with an increased risk of ARV infection. The season of grow-out was associated with the ARV mean titre, with significantly lower titres in the winter compared to the spring, summer, and autumn. Differences in ARV mean titres affiliated with feed mills supplying feed to the flocks were also identified.

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CHAPTER 1: INTRODUCTION, LITERATURE REVIEW, AND THESIS OBJECTIVES

INTRODUCTION

Avian reoviruses (ARVs) are members of the *Orthoreovirus* genus in the family *Reoviridae*. The name "reovirus" derives from the acronym for Respiratory Enteric Orphan, because they were first isolated from these sites in humans with initially no apparent association with disease.

In chickens, the most recognized form of ARV associated diseases, and also a significant cause of lameness, is tenosynovitis (1). Tenosynovitis, also known as viral arthritis, is predominantly a problem in broiler chickens, and is characterized by swelling in the hock joint. Depending on the degree of severity of the inflammation, an affected bird may be unable to move towards feed and water resulting in poor growth or death.Birds that survive to slaughter may be downgraded because of inflamed hock joints.Avian reoviruses are also associated with a variety of other diseases in chickens, such as respiratory and enteric disease, hydropericardium, pericarditis, myocarditis, and hepatitis (1).Many of these diseases affect chicken producers financially due to production losses, due to poor feed conversion, increased culling, and carcass condemnations.

Thus, in modern agricultural industries where high-tech production and cutting-edge research go hand-in-hand to optimize productivity, control and prevention of these production-limiting diseases must be of utmost importance. This literature review focuses on reovirus infections in broiler chickens, including the characteristics of the virus, modes of transmission and routes of exposure, traditional methods of prevention and control, laboratory methods for detecting ARV, the prevalence of ARV globally, and risk factors for ARV.

LITERATURE REVIEW

Characteristics of the virus

Avian reoviruses are heat resistant, stable in a wide spectrum of pH, and are resistant to disinfectants commonly used in poultry houses (e.g., 2% formaldehyde, 2% phenol) (2). They can survive in an environment of pH 3.0 to 9.0, and are active at up to 56 °C for up to 24 hours. Thus, complex measures are required to prevent ARV from entering poultry barns. Despite the early identification of the characteristics of ARV, their survivability in poultry barns had not been studied until the early 2000s. A study of ARV's survivability on materials commonly used in poultry barns showed that ARV can survive for up to 10 days on feathers, wood shavings, glass, rubber, and galvanised metal, and for 10 weeks in water (3). However, there have been no studies conducted to determine if there is an association between contaminated fomites and ARV infection. The survivability of the pathogen in water poses a threat because once a drinking water system is contaminated with ARV, it can remain a source of infection for up to 10 weeks, which is long enough to infect the next flock raised in the barn.

Modes of transmission and routes of exposure

Experimental studies have shown that ARV can be transmitted both vertically and horizontally(4-7). Day-old chicks are most susceptible to ARV infection(1). Therefore, if the parent flock is not vaccinated or exposed in the field before the onset of production, the broiler progeny will have no maternal antibodies and can become infected at an early age from other chicks that may be shedding the virus, or from the environment via the faecal-oral route (8). Additional modes of transmission of ARV include infection through broken skin of the foot pad and the respiratory route (5).

Methods of control

As maintaining an ARV-free flock is difficult to achieve, the principal methods to control ARV infection are strict biosecurity, good management practices, and vaccination. Implementation of strict biosecurity and good management practices includes minimizing entry into the barn, practicing all-in-all-out management, effective cleaning and disinfecting of the barn, and good record keeping. Because ARVs are resistant to disinfectants commonly used in poultry houses (2), effective cleaning and disinfection require a careful consideration in choosing barn cleaning products (9).

Generally, ARV vaccination programs consist of the use of live and killed vaccines in breeder flocks(10). Most commonly used vaccines are based on the S1133 strain (1). Eidson et al. (10) showed that the progeny of vaccinated breeder hens were resistant to ARV challenge via the oral/ subcutaneous route (10). However, due to the variety of pathogenic strains of ARV in the field, the efficacy of currently available vaccines may be limited to viruses that are similar to the vaccine strain(10).

Laboratory methods for detecting ARV

A number of laboratory methods have been developed to detect antibodies against ARV. These include immunofluorescence, serum neutralization, immunodiffusion, and enzyme-linked immunosorbent assay (ELISA). Although these traditional methods are successful in detecting ARV infection, they are often time consuming and laborious, with the exception of ELISA, are not well suited for screening a large number of serum samples(11).One limitation of ELISAs is that theycannot distinguish ARV genotypes, which can be useful for epidemiological baseline surveys in poultry flocks, and for establishing effective biosecurity measures and vaccination programs. Additionally, at present, commercially available ELISAs cannot distinguish between antibodies from vaccinated and infected birds.

However, an ELISA has recently been developed to distinguish vaccine ARV and wildtype ARV antibodies. Xie et al. (11) usednon-structural proteins as antigens to detect specific antibodies for ARV in serum samples from infected birds. Although these researchers were able to differentiate between infected and vaccinated sera, the sensitivity and specificity of the method were limited (61.1 to 88.9% of serum samples from infected chickens tested positive, and 0 to 6.7% of serum samples from the vaccinated chickens tested positive).

In addition to serological testing, virus isolation and PCR can be used to detect ARV in various types of tissues. Because the virus is ubiquitous, detection of virus in intestinal samples may not be useful for determining an association with tenosynovitis (1). Another useful laboratory diagnostic tool for ARV detection is PCR. According to Liu et al., a combination reverse-transcriptase PCR and restriction enzyme fragment length polymorphism enables characterization of ARV isolates(12), which can be beneficial to molecular epidemiological investigations.

Prevalence of ARV

A study by Ide and Dewitt (13) reported a high incidence of serological reactors to ARV using the plaque neutralization test and the agar gel precipitation (AGP) test. Twelve of 14 broiler breeder flocks in Nova Scotia tested positive. The survey was carried out prior to any ARV vaccine use in Canada, suggesting that the occurrence of reovirus was due to natural infection. Although the study reported a high seroprevalence of ARV in the breeder flocks, relatively few clinical signs of tenosynovitis were observed in progeny flocks (0.3 to 1.9%). The finding suggested the existence of different ARV strains in the population, variation in pathogenicity of the strains, and protection of the progeny flocks due to maternal immunity.

In 1984, Robertson et al. (14) conducted an investigation of ARV prevalence in commercial chickens with tenosynovitis in Australia. Tissue samples of tendon, bursa, spleen,

pancreas, and rectum were collected from 97 clinically affected chickens and 62 clinically normal chickens. Avian reovirus was isolated from a number of tissues collected: tendon (11/96 samples), bursa (0/11), spleen (3/13), pancreas (6/6), and rectum (5/6). The virus was also isolated from the rectal tissues of clinically normal chickens (49/62). Also, serum samples were collected from 10 different broiler breeder farms in Victoria, New South Wales, and Western Australia to determine the prevalence of ARV antibody using the serum neutralization test and the AGP test. The AGP test results showed 90%, 100%, and 80% of the flocks tested positive in Victoria, New South Wales, and Western Australia, respectively. One limitation of the study is that the origin of clinically affected chickens was not specified; therefore, the result of ARV isolation might not be representative of the prevalence of tenosynovitis in Australia. Such high seropositivity of ARV alone in the Australian broiler breeder population may be attributed to the use of ARV vaccines. High seroprevalence of ARV does not suggest a causative association between the presence of ARV and development of tenosynovitis.

A surveillance study was carried out in Iran from 2004 to 2005 to determine the seroprevalence of ARV in broiler flocks in Tehran province (15). Serum samples were collected from 72 randomly selected flocks at the age of slaughter. AnELISA was used to detect antibodies against ARV in the serum samples. Results of the ELISA test revealed 98.3% seropositivity for ARV antibodies in the samples, suggesting a high prevalence of ARV infection in poultry flocks of Tehran. The strength of this study lies in the epidemiologically sound design of the survey: careful sample size calculation, randomization of sample collection, accounting for clustering, and identification of the weakness of currently available methodology, therefore avoiding potential misclassification bias. One weakness of the study was the limited means to distinguish between ARV vaccine and natural ARV antibodies.

A team of Belgian researchers conducted a study on 70 broiler farms that showed signs of lameness, and/ or poor growth or increased mortality between August 2001 and October 2006 (16). Samples and detailed case history data were collected to evaluate the prevalence of ARV in the affected flocks. The presence of ARV was confirmed in 22 broiler flocks; among them, 21 flocks were affected with Enteric Reovirus Strain (ERS), which has been associated with malabsorption syndrome in broiler chickens (16).

Risk factors for ARV

Host age

Studies suggest thatthe impact of ARV infection is age-linked. As demonstrated in1984 by Jones et al., day-old chicks are at the highest risk of ARV infection due to their inability to mount an effective immune response against the pathogen. In this experimental study, day-old chicks were more susceptible to induced tenosynovitis and thus showed more severe clinical signs of ARV infection than chicks infected at older ages (8). This finding is also supported by Montgomery et al. (17).

Host immune status

The principal method to control ARV infection is vaccination. This is achieved either by active or passive immunity. Usually, live and killed vaccines are used in broiler breeders to provide passive immunity for the broiler progeny; active immunity is acquired by directly vaccinating broiler chicks. Mukiibi-Muka (1997) compared the effectiveness of passive and active immunity at protecting one day old chicks against ARV infection, and found that vaccination (active immunity) was less effective (18).

Strains of ARV

Many strains of ARV have been identified in both chickens and turkeys around the world since its isolation by Fahey and Crawley in 1954 (19). Commonly reported pathogenic ARV

strains include, but are not limited to: S1133, UMI 203, Reo 25, WVU 2937, 2408, S1733, CO8, ARV 305, ss412, ARV CU-98, ARV NL120 98M, ARV 176, and ARV 138 (20). The isolated ARV strains show a broad spectrum of pathogenicity, which can be challenging to reproduce experimentally (21, 22). In addition, approximately 80% of isolated ARV strains are non-pathogenic (1).

Co-infection

Avian reovirus associated diseases may be a result of co-infection with other infectious pathogens. A number of studies reported that infectious bursal disease virus, chicken anaemia virus, and *Mycoplasma synoviae*have been found in the joint lesions of chickens with tenosynovitis (23-26).

THESIS OBJECTIVES

Despite the high prevalence and widespread nature of the virus, to our knowledge, there have been no epidemiological studies conductedto identify risk factors for ARV. At present, there is insufficient information on the prevalence, distribution, or risk factors of ARV in the Ontario broiler industry. The first objective of the thesis was to establish the baseline flock-level prevalence, geographical distribution, and seasonal variation of ARV among commercial broiler chicken flocks in Ontario during grow-out.The second objective wasto identify biosecurity and management practices associated with the presence of ARV. The knowledge gained from this thesis is expected to assist the broiler industry tounderstand the effectiveness of current biosecurity and management practices on the risk of ARV infection, and develop disease control strategies.

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CHAPTER 2: FLOCK-LEVEL PREVALENCE, GEOGRAPHICAL DISTRIBUTION, AND SEASONAL VARIATION OF AVIAN REOVIRUS AMONG COMMERCIAL BROILER CHICKEN FLOCKS IN ONTARIO

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ABSTRACT

Avian reovirus (ARV) is an economically significant pathogen of broiler chickens. Our objective was to determine the prevalence, geographical distribution, and seasonal variation of ARV infection among commercial broiler chicken flocks in Ontario, Canada during grow-out. A cross-sectional study of 231 broiler flocks was conducted from July 2010 to January 2012. Each month, approximately equal numbers of randomly-selected flocks were enrolled. Samples were collected at six processing plants representing 70% of Ontario's broiler processing. Fifteen blood samples, 15 whole intestines, and 15 cloacal swabs per flock were collected at slaughter; ELISA and PCR were used to determine a flock's ARV exposure status. Avian reovirus prevalence in the study population was91% (95% confidence interval:86% to 94%). Using aunivariable logistic regression model, statistically significant associations were identified between ARV presence and geographic district ($p = 0.012$). Using a univariable exact logistic regression model, it was found that the odds of ARV presence were significantly lower in the summer compared to the other seasons ($p< 0.001$). There was no association between flock mortality and flock ELISA mean titre or PCR status.

INTRODUCTION

Avian reoviruses (ARVs) are members of the *Orthoreovirus* genus in the family *Reoviridae*. Avian reoviruses are associated with a variety of diseases in chickens, such as

respiratory and enteric disease, hydropericardium, pericarditis, myocarditis, and hepatitis (1). However, the most recognized form of ARV-associated diseases, and also a significant cause of lameness, is tenosynovitis (1).

Tenosynovitis, also known as viral arthritis, is predominantly a problem in broiler chickens, and results in swelling of the hock joint(s). Depending on the severity of the inflammation, affected birds are unable to move toward feed and water resulting in poor growth, culling, or death. Birds that survive to slaughter may be downgraded because of inflamed hock joints. This is a serious economic concern for broiler producers due to the costs associated with feed, labour, and lost production.

Avian reovirus infection in broiler chicken flocks appears to be high. Owoade et al (2006) reported a seroprevalence of 41.0% among Nigerian broiler flocks (2), whereas studies conducted in Iran, and four western provinces in Turkey,reported higher seroprevalence (98.3%, 70.6%, 77.2%, 76.8%, and 75.9%, respectively) (3, 4).

At present, there is insufficient information on the prevalence or distribution of ARV in the Ontario broiler industry. An understanding of the baseline prevalence is the first step toward determining the effectiveness of current biosecurity and management practices on the risk of ARV infection, and developing disease control strategies. Thus, the objectives of this study were to establish the baseline flock-level prevalence, geographical distribution, and seasonal variation of ARV among commercial broiler chicken flocks in Ontario during grow-out.

MATERIALS AND METHODS

Sampling

This study was part of a larger project designed to estimate the flock-level prevalence and risk factors for 13 pathogens of poultry health significance in the Ontario broiler industry. The

sampling frame was all broiler producers in Ontario that were contracted with six major processing plants (five federal and one provincial). These plants represent approximately 70% of broiler chickens processed in the province. Sample size estimates were based on identifying risk factors. Thus, using 95% confidence, 80% power, and a difference of 20% between the proportions of exposed and unexposed flocks with an estimated baseline prevalence of 20% (which was assumed to be adequate for all 13 pathogens), the target sample size to simultaneously evaluate multiple risk factors was 240 flocks. Approximately equal numbers of randomly-selected flocks were enrolled every 4 weeks during the sampling period—July 2010 to January 2012—to account for potential seasonal variation in pathogen prevalence. The number of flocks sampled from each processing plant was proportional to the plant's market share of Ontario's broiler processing. The days on which each plant was visited during each 4-week period was randomized using MiniTab 14 statistical software (Minitab Inc., State College, Pennsylvania, USA).For each sampling day, one flock was randomly-selected using numbered coins from the list of flocks scheduled to be slaughtered that day, and the corresponding producer was phoned and invited to participate in the study. The number of flocks sampled per farm was limited to one; if more than one flock was selected from the same farm, only the first flock was considered for inclusion in the study. Flocks originating from Québec were excluded from the study.

The number of samples required per flock was determined using an *a priori* within-flock prevalence estimate of 15% and a confidence level of 90%. This *a priori* estimate was deemed sufficient to detect all pathogens of interest in the context of the larger project.At the processing plants, 15 blood samples, 15 cloacal swabs pooled into three samples (5 swabs per sample), and 15 whole intestines (duodenum to cloaca) were collected conveniently from each flock.Each

blood sample contained blood from one or more birds depending on the line-speed and set-up of the plant. If a flock was shipped by more than one truck, the number of samples collected from each truckload of birds was approximately equal. For example, if five trucks were used to ship a flock, three samples were collected from each truckload of birds to ensure representative sampling of the flock.

Sample Processing

Samples were transported in coolers with ice packs to the Animal Health Laboratory, Guelph, Ontario. The samples were then further processed before submission for laboratory testing; for caecal tonsil tissue samples, three pools of five caecal tonsil tissues were collected from whole intestines.Instruments were autoclaved between flocks.

Laboratory Testing

A commercially available enzyme-linked immunosorbent assay (ELISA) kit (IDEXX Laboratories, Inc., Westbrook, Maine, USA) was used to detect ARV antibodies in 15 pooled blood samples. The ELISA procedure was carried out as recommended by the manufacturer. Three pools of cecal tonsils, and 3 pools ofcloacal swabs were used for virus isolation in cell culture and then polymerase chain reaction was carried out on cell culture fluids to detect viral shedding.Virus isolation was conducted in Leghorn male hepatoma cells obtained from American Type Culture Collection (ATCC#CRL-2117) as described previously (5).Total nucleic acids for ARV-specific real-time PCR were extracted using MagMAX-96 Viral RNA Isolation Kit in a MagMAX Express-96 Magnetic Particle Processor (Applied Biosystems Inc., Foster City, California, USA) according to the manufacturer's instructions. The PCR amplification was carried out in 25 µL reactions in a LightCycler 480 Real-Time PCR System (Roche, Laval, Québec, Canada) using AgPath-ID One-Step RT-PCR Kit (Applied Biosystems).

Data analysis

The total number of samples tested per flock using PCR was 6 (3 pooled samples of caecal tonsil tissues and 3 pooled samples of cloacal swabs); if ≥ 1 of 6 samples tested positive, the flock was considered to be PCR-positive. Criteria used to classify flocks as positive or negative for ARV based on ELISA and PCR results are shown in Table 2.1. For ELISA mean titres, the cut-point used (396) was that specified in the IDEXX reference guide.Titre groups provided by IDEXX are shown in Appendix 1. Flocks were further stratified by ELISA and PCR test results and the average flock mortality per stratum was estimated (Table 2.2). For each flock, mortality was calculated as the number of chicks placed minus the number of birds shipped, divided by the number of chicks placed, and multiplied by 100. For each stratum, average mortality was calculated as the sum of flock mortality in the stratum divided by the number of flocks in the stratum. A bi-variable linear regression model was used to determine if there was an association between flock mortality (dependent variable) and flock ELISA mean titre (categorical) and flock PCR status (dichotomous) (Table 2.2); model assumptions of normality and homoscedasticity were evaluated visually using a normal quantile plot and a plot of the standardized residuals against the predicted outcome, respectively.

To explore potential seasonal variation in ARV prevalence, each flock was categorized into one of four seasons (spring = March 21st to June 20th; summer = June 21st to September 21st; autumn = September 22nd to December 20th; winter = December 21st to March 20th). For example, a flock was considered to have beenraised in the summer if the half age of the flock (19 days on average in the study population) was before the first calendar day of autumn. Half age of the flock was chosen because broiler breeders in Ontario are vaccinated against ARV, and maternal immunity is expected to decrease after approximately 14 days of age, leaving the chicks

vulnerable to infection if exposed to the virus.A univariable exact logistic regression model was used to determine if there were statistically significant differences in prevalence between seasons. For the exact logistic model, a new variable was generated to investigate the statistical significance between ARV and season. If a season had 100% of ARV prevalence, the season was categorized as "other seasons"; spring, autumn, and winter were collapsed into one category andcompared to summer, which had the lowest prevalence and thereforewas chosen as the referent category.

A choropleth map of the geographical distribution of ARV prevalence in Ontario was created using ArcGIS 9 (Esri, Redlands, California, USA). A reference map of Ontario's nine broiler districts (administrative areas) was obtained from the Chicken Farmers of Ontario (CFO) and used to create a prevalence map;Jenk's natural breakdown classification method was used to create prevalence classes in ArcMap®. A univariable logistic regression model was used to determine if there were statistically significant differences in prevalence between districts. All statistical analyses ($\alpha = 0.05$, two-tailed) were performed using Stata Intercooled version 10 (StataCorp, College Station, Texas, USA). The fit of each logistic regression model was assessed using the Pearson chi-square goodness-of-fit test.

RESULTS

In total, 231flocks were sampled between July 2010 and January 2012. For blood samples, 1 flock had 10 blood samples collected, 5 had 14 collected, and the remainder (225, 97.4%) had 15 samples collected.

Characteristics of the study population

Fourproducers that allowedsample collection at the processing plant did not complete an interview. Consequently, farm/ flock-level data were available for 227 flocks. The mean and

median weight of birdsat processing was 2.2 kg (range: 1.7to 3.1 kg). The mean and median flock age at shipment was 38 days (range: 31 to 53 days). The mean mortality per flock was 3.5% (range: 0.3 to 12.7%); this included both natural death and culling.The median flock size was 25,092 birds (range:7,242 to 104,040 birds;flock sizes were available for 200 flocks). The number of flocks raised using an all-in-all-out system of production at the flock level (i.e., all birds within a flock were placed on the same date and also shipped for processing simultaneously) was 223 of 227 flocks (98.2%). The number of barns the flock was raised in varied: one barn (168/227 flocks; 74.0%), two barns (46 flocks; 20.3%), three barns (8 flocks; 3.5%), and four barns (5 flocks; 2.2%).

Avian reovirus prevalence

Two hundred and nine of 231 flocks (90.5%; 95% CI 86.8 to 94.3%) were positive for ARV. The number and percentage of flocks per classification criterion are shown in Table 2.1.The ELISA mean titres ranged from 1 to 5,222; 120 flocks (51.9%) tested positive and 111 flocks (48.1%) tested negative on PCR (Table 2.2). The average flock mortality stratified by ELISA mean titre and PCR status ranged from 3.0 to 4.8% (Table 2.2); the stratum-specific ranges were variable in both the PCR-positive and –negative groups, with very high flock-level mortality on some farms. There was no association between flock mortality, and flock ELISA and PCR results, and the assumptions of linear regression were met (Table 2.3).

The ARV prevalence by season was 100% in the spring (29/29), autumn (84/84) and winter (39/39), and72.2% (57/79) in the summer.A model using season as a categorical independent variable could not be built due to perfect prediction (100% prevalence of ARV in the spring, autumn, and winter).The results of the exact logistic regression showed thatother seasons were at greater odds of testing positive to ARV compared to summer (odds ratio[median

unbiased estimate]: 81.26; exact $p < 0.001$; exact 95% CI [13.83, positive infinity], Table 2.3).The prevalence of ARV by broiler district ranged from 80% (district 5) to 100% (district 8)(Table 2.4, Figure 2.1).A model using district as a categorical independent variable (districts 1 through 9) could not be developed because district 8, which had a prevalence of 100%, dropped out of the model due toperfect prediction. Therefore, an alternative categorical variable representing districts was created using the same prevalence classes used to generate the choropleth map (Figure 2.1). The resultant model showed that ARV presence varied by prevalence class (LRT χ^2= 12.86, p = 0.012; Table 2.3);the presence of ARV was significantly lower in districts with an ARV flock-level prevalence of 80 to 82% (district 5) (OR = 0.06, p = 0.017) and districts with a prevalence of 83 to 86% (districts 4, 3, and 1) (OR = 0.08, p = 0.015) compared to districts with a prevalence of96 to 100% (districts 2 and 8). The Pearson's χ^2 test was not significant (p >0.99).

DISCUSSION

Avian reovirus has been found on many poultry farms suffering from tenosynovitis, malabsorption syndrome, and enteric/respiratory diseases (1). These diseases result in significant economic losses; however, todate, there is insufficient information on the prevalence and distribution of the virus in the commercial broiler population in Canada. This study captured the baseline prevalence of ARV among a large sample of randomly-selected broiler flocks at processing in Ontario between July 2010 and January 2012. Although ARV infection was common (90.5%) among broiler flocks in Ontario, this was not synonymous with ARV-associated diseases. Our findings are similar to the reported prevalence in Iran (98.3%) (3) and Turkey (70.6 to 77.2%) (4), and higher than the prevalence reportedin Nigeria (41.0%) (2). Classification criteria used to determine flock status (positive/negative) in both the Nigerian and

Iranian studies were different from our study, which make it difficult to compare the findings. Further, the following aspects were different in our study design/population: the age of broilers at sampling, vaccination of breeders, and antibody titre thresholds for reovirus seropositivity. Similar to our study, sampling in the Iranian study was conducted at the time of slaughter, although the age was not specified. The study conducted in Nigeria sampled blood from 1 day old to 8 week old broilers. In contrast to our study, in which all domestic broiler breeder flocks are vaccinated for ARV several times as pullets, including at least two live vaccines and two killed vaccines, the parent flocks in the Iranian and Nigerian studies were not vaccinated against reovirus. Furthermore, the Nigerian and Iranian studies used only one test (ELISA) to determine the flocks' seropositivity; however, the mean titre threshold used in the Nigerian study was higher than our study (1,351 vs. 396), and the threshold used in the Iranian study was not specified.

In our study population, the proportion of flocks that were PCR positive was approximately equal to the proportion that was PCR negative. Further, the distribution of ELISA mean titres was similar between the PCR-positive and PCR-negative flocks. Interpretation of the mean titre and PCR results could indicate when(early vs. late) exposure likely occurred.For example, flocks with high mean titres were likely exposed to ARV earlier during the grow-out period, therefore had high antibody titres at the time of slaughter, yet may or may not have been shedding virus at that time. In contrast,PCR-positive flocks with low mean titres were likely exposed to ARV late in the grow-out period, and therefore had not mounted a full immune response at the time of slaughter, yet were shedding virus that could be detected on PCR. Such detailmight be important when exploring associations between ARV exposure and production data.

The mean mortality in our study population was consistent with the provincial average; the CFO reported that the average mortality for the province between 2005 and 2009 was 4.0% (range: 3.6 to 6.1%) for all slaughter ages and weights. There was no statistically significant association between flock mortality and the flock's ELISA mean titre and PCR status, suggesting that there was no direct relationship between the antibody titres and mortality in this baseline surveillance study. This finding might be related to a lack of differentiation between pathogenic and non-pathogenic strains. Conversely, because we could not differentiate between natural mortality and culling, mortality in our study might be a surrogate for poor management or heavy culling for ARV-associated diseases or other diseases, as suggested by the widely varied flock-level mortality for some farms in several of the low to mid-titre strata.

Randomization resulted in excellent representation of the distribution of broiler production in the province (6). Although the prevalence of ARV was very high across the province, statistically significant differences in prevalence betweendistrict classes were identified. The variation in the geographical distribution of ARV could be due to localized differences in risk factors, such as environmental challenges, the density of farms, early mortality associated with *E.coli*, or subtle differences in management and biosecurity practices among districts. The virus can survive under farm conditions for 12-15 weeks—it is heat resistant, stable in a wide spectrum of pH (3.0 to 9.0), and resistant to disinfectants commonly used in poultry houses (7). This environmental resistance of the virus is likely one of the reasons for the high prevalence across the province.

To our knowledge, this is the first study that has investigated the seasonal variation in ARV prevalence. Avian reovirus was common in all seasons. Meulemanns and Halen (1982) found that ARV was active at temperatures up to 50°C, indicating the hardiness of the virus (8).

We found that ARV was less prevalent in the summer compared to other seasons. It is possible that factors that vary among seasons, such as cleaning and disinfection practices, or ventilation, might have contributed to the minor seasonal variation in prevalence. In Ontario, the CFO's On-Farm Food Safety Assurance Program dictates that broiler barns must be disinfected at least once per year. Given the climate in Ontario, it is possible that many producers carry out this task in the summer when ambient temperatures are more favourable for thorough cleaning and disinfection.

Avian reovirus has been associated with several poultry diseases. However, the pathogenesis is unclear and its presentation is likely influenced by other factors. Commercial ELISA cannot distinguish pathogenic strains of reovirus from non-pathogenic strains (9); therefore, it is not known what percentage of flocks in our study was exposed to pathogenic strains.

Our study findings indicate that ARV is common among commercial Ontario broiler flocks in all seasons and in all broiler districts. Investigation of associations between ARV and aspects of flock management, such as biosecurity and management practices are warranted, as are associations between ARV and flock production parameters.

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Table 2.1. Criteria used to classify commercial broiler chicken flocks sampled at processing between July 2010 and January 2012 in Ontario, Canada, as positive or negativefor avian reovirus based on enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) results, and the number and percentage of flocks per criterion (n = 231 flocks)

ELISA[a]	**PCR positive**[b]	**PCR negative**
Mean titre > 396	*Flock ARV positive* 76 (32.9%)	*Flock ARV positive* 70 (30.3%)
Mean titre ≤ 396 and % samples ≥ 10%[c]	*Flock ARV positive* 31 (13.4%)	*Flock ARV positive* 19 (8.2%)
Mean titre ≤ 396 and % samples < 10%[d]	*Flock ARV positive* 13 (5.6%)	*Flock ARV negative* 22 (9.5%)

[a]The cut-point used for the ELISA mean titre was that specified in the IDEXX (IDEXX Laboratories, Inc., Westbrook, Maine, USA) reference guide.

[b]The total number of samples tested per flock using PCR was 6 (3 pooled samples of caecal tonsil tissues and 3 pooled samples of cloacal swabs); if ≥ 1 of 6 samples tested positive, the flock was considered to be PCR-positive.

[c]At least 10% of the blood samples were in profile group 1 (titres of 397 to 999) or higher.Profile groups are based on titre levels; asthe level of antibody titre in a sample increases, the group number increases. The profile groups for this test range from 0 to 18.

[d]The percentage of blood samples in profile group 1 or higher was less than 10%.

Table 2.2.Number of commercial broiler chicken flocks sampled at processing between July 2010 and January 2012 in Ontario, Canada stratified byenzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction(PCR)test results for avian reovirus (n = 231 flocks); the average flock mortality[a] per stratum is provided

ELISA mean titre	PCR positive[b]	Average (range) flock mortality (%)	PCR negative	Average (range) flock mortality (%)
5,000 – 5,999	1	3.1	0	-
4,000 – 4,999	2	3.2 (0.8 – 5.7)	0	-
3,000 – 3,999	2	3.0 (2.4 – 3.5)	2	3.0 (2.9 – 3.2)
2,000 – 2,999	8	3.9 (2.3 – 7.6)	5	4.8 (2.2 – 9.4)
1,000 – 1,999	18	3.6 (0.3 – 6.3)[c]	22	3.5 (1.7 – 6.4)[g]
500 – 999	31	3.6 (0.6 – 5.8)[d]	26	3.6 (1.3 – 7.6)[h]
397 - 499	14	3.3 (0.8 – 8.9)[e]	15	4.4 (2.1 – 6.4)[i]
0 - 396	44	3.2 (1.9 – 5.5)[f]	41	3.4 (0.6 – 12.7)[j]

[a]For each flock, mortality was estimated as the number of chicks placed minus the number of birds shipped, divided by the number of chicks placed, and multiplied by 100. For each stratum, average mortality was calculated as the sum of flock mortality in the stratum divided by the number of flocks in the stratum.

[b]The total number of samples tested per flock using PCR was 6 (3 pooled samples of caecal tonsil tissues and 3 pooled samples of cloacal swabs); if ≥ 1 of 6 samples tested positive, the flock was considered to be PCR-positive.

[c]Sixteen of 18 flocks used in calculation due to missing mortality data for two flocks.

[d]Twenty-three of 31 flocks used in calculation due to missing mortality data for eight flocks.

[e]Eleven of 14 flocks used in calculation due to missing mortality data for three flocks.

[f]Thirty-seven of 44 flocks used in calculation due to missing mortality data for seven flocks.

[g]Eighteen of 22 flocks used in calculation due to missing mortality data for four flocks.

[h] Twenty-three of 26 flocks used in calculation due to missing mortality data for three flocks.

[i]Fourteen of 15 flocks used in calculation due to missing mortality data for one flock.

[j]Thirty-eight of 41 flocks used in calculation due to missing mortality data for three flocks.

Table 2.3.Final regression models for the associations between flock mortality and flock avian reovirus (ARV) ELISA[a] and PCR[b] test results (linear), ARV presence and season of grow-out (logistic), and ARV presence and district of grow-out (logistic), in commercial broiler chicken flocks in Ontario

Linear regression: flock mortality and flock ARV ELISA and PCR test results (n = 198 flocks)					
Variable		**Coefficient**	**95% CI of coefficient**	**p-value (t-test)**	**p-value (partial F-test)**
Flock ELISA mean titre					0.634
5,000 – 5,999		2.70	(-0.60, 6.00)	0.108	
4,000 – 4,999		0.96	(-1.39, 3.30)	0.421	
3,000 – 3,999		0.34	(-1.34, 2.02)	0.687	
2,000 – 2,999		-0.21	(-1.23, 0.81)	0.686	
1,000 – 1,999		0.09	(-0.59, 0.77)	0.794	
500 – 999		0.11	(-0.50, 0.73)	0.716	
397 – 499		-0.38	(-1.15, 0.39)	0.334	
0 - 396		Referent			
Flock positive to ARV on PCR[c]					
Yes		-0.16	(-0.63, 0.32)	0.519	
No		Referent			
Intercept		3.62	(3.15, 4.10)	< 0.001	
Significance of overall model					p-value (F-test) = 0.716 R^2 = 0.028
Exact logistic regression: ARV presence and season (n = 231 flocks)					
Variable	**Odds ratio[d]**		**Exact 95% CI of OR**	**Exact p-value**	

Season Other seasons Summer	 81.26 Referent		(13.83, positive infinity)	 < 0.001	
Logistic regression: ARV presence and district (n = 229 flocks)					
Variable	**Odds ratio**	**Coefficient**	**95% CI of coefficient**	**p-value (Wald's test)**	**p-value (Likelihood ratio test)**
Classes of district[e]					0.012
Prevalence class 1	0.06	-2.86	(-5.21, -0.52)	0.017	
Prevalence class 2	0.08	-2.55	(-4.61, -0.49)	0.015	
Prevalence class 3	0.13	-2.01	(-4.32, 0.29)	0.087	
Prevalence class 4	0.19	-1.68	(-4.13, 0.76)	0.177	
Prevalence class 5	Referent				
Intercept		4.25	(2.27,6.22)	<0.001	

[a] ELISA = enzyme-linked immunosorbent assay.

[b] PCR = polymerase chain reaction.

[c] At least one of six pooled caecal tonsil tissue or pooled cloacal swab samples tested positive to ARV on PCR.

[d] Medianunbiased estimate.

[e]The Chicken Farmers of Ontario, a producer-run, non-profit organization representing registered Ontario chicken producers partitions Ontario into nine administrative / geographical areas referred to as broiler districts. Prevalence class 1 represents districts with an ARV flock-level prevalence of 80 to 82% (district 5), class 2 represents districts with a prevalence of 83 to 86% (districts 4, 3, and 1), class 3 represents districts with a prevalence of 87 to 91% (districts 6 and 9), class4 represents districts with a prevalence of92 to 95% (district 7), andclass5 represents districts with a prevalence of96 to 100% (districts 2 and 8).

Table 2.4. Flock-level avian reovirus prevalence among commercial broiler chicken flocks sampled at processing between July 2010 and January 2012in Ontario, Canada by broiler district (n = 231 flocks)

District	1	2	3	4	5	6	7	8	9
Prevalence	18/21[a] (85.7%)	37/38 (97.4%)	38/45 (84.4%)	15/18 (83.3%)	12/15 (80.0%)	9/10 (90.0%)	27/29 (93.1%)	33/33 (100%)	20/22 (90.9%)

[a]The denominator indicates the number of flocks sampled per district.

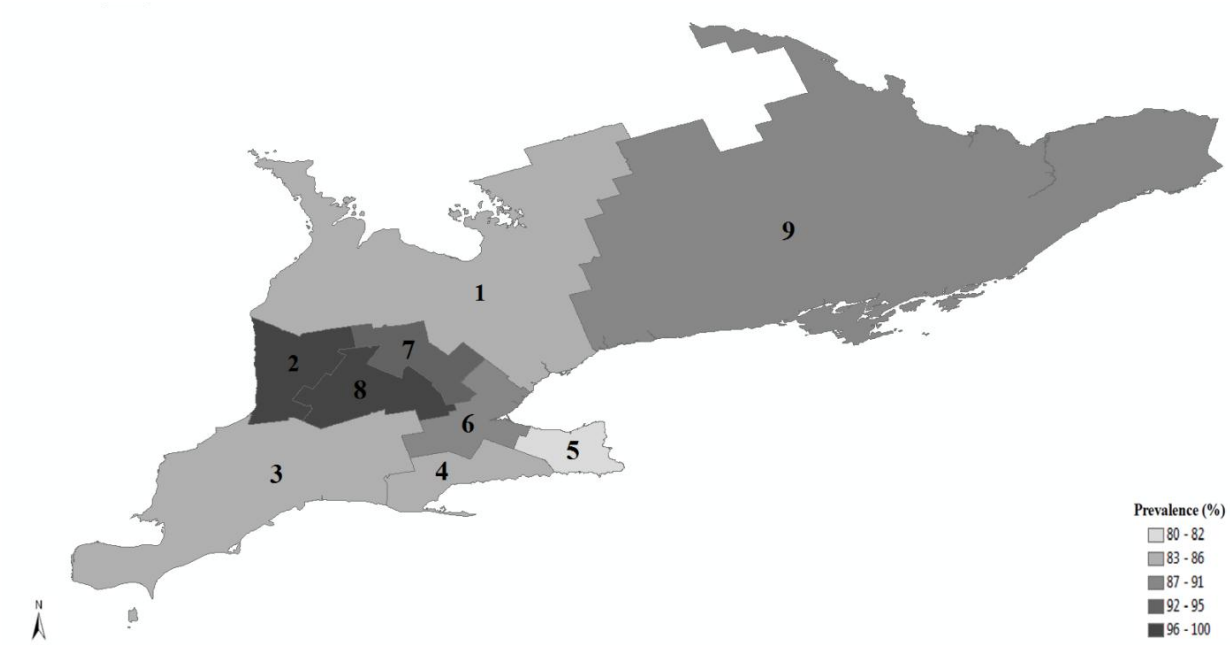

Figure 2.1. Flock-level avian reovirus prevalence among commercial broiler chicken flocks sampled at processing between July 2010 and January 2012 in Ontario, Canada by broiler district (n = 231 flocks). The map was produced using ArcGIS® 9 (Esri, Redlands, California, USA)

CHAPTER 3: FLOCK-LEVEL RISK FACTORS ASSOCIATED WITH AVIAN REOVIRUS AMONG COMMERCIAL BROILER CHICKEN FLOCKS IN ONTARIO

Prepared for submission to the*Canadian Journal of Veterinary Research*

ABSTRACT

Avian reovirus (ARV) is associated with many production-limiting diseases in the broiler chicken industry. This research focused on identifying biosecurity and management practices associated withthe presence of ARV among commercial broiler chicken flocks in Ontario. Biological samples and flock/ farm-level management data were collected fromJuly 2010 toJanuary 2012. Our data were modelled in two ways: linear regression using the ELISA mean titre of the flock as the dependent variable, andlogistic regression using the PCR status of a sample (positive or negative for ARV) as the dependent variable with flock as a random intercept. In both models, co-infection with chicken anemia viruswas associated with an increased risk of ARV. The season of grow-out was associated with ARV mean titre, with significantly lower titres in the winter compared to the spring, summer, and autumn. Differences in ARV mean titres between feed mills supplying feed to the flocks were also identified.

INTRODUCTION

Avian reovirus infection has been associated with a variety of diseases in chickens, including tenosynovitis, respiratory and enteric disease, hydropericardium, pericarditis, myocarditis, and hepatitis (1). Avian reovirus infections are widely spread in poultry populations worldwide (Chapter 2).

In an experimental study, ARVs survived for up to 10 days on feathers, wood shavings, glass, rubber, and galvanised metal, and for 10 weeks in water (2). Avian reovirus can be transmitted horizontally and vertically, and is resistant to disinfectants commonly used in poultry barns (3-7). Horizontal modes of ARV transmission include exposure through broken skin of the foot pad (6), or via the oral (1) or respiratory route (6). Although transmission through the oral route is possible, one day old chicks are more susceptible to ARV infection by the respiratory route (1). Traditionally, the principal methods to control ARV infection in broilers have been strict biosecurity/ management practices, and vaccination of parent flocks to confer maternal immunity to the chicks.Factors that have been linked to ARV-associated disease include host age, host immune status, strains of ARV, and co-infection with other pathogens (7-10). Jones (2008) demonstrated that day old chicks are more susceptible to ARV infection than older chicks (1). Mukiibi-Muka (1997) compared the effectiveness of passive and active immunity at protecting one day old chicks against ARV infection, and found thatdirect vaccination (active immunity) was less effective (11). Commonly reported pathogenic ARV strains include, but are not limited to S1133, UMI 203, Reo 25, WVU 2937, 2408, S1733, CO8, ARV 305, ss412, ARV CU-98, ARV NL120 98M, ARV 176, and ARV 138 (12). Many strains of ARV with a broad spectrum of pathogenicity have been identified (12-14). ARV has been linked to worsen disease conditions caused by chicken anaemia virus (CAV), and *Escherichia coli*(8,10), and exposure to infectious bursal disease virus (IBDV) has been associated with increased severity of tenosynovitis caused by ARV, suggesting co-infection of ARV and the listed pathogens (7,9).

Despite the high prevalence and widespread nature of the virus, to our knowledge, there have been no epidemiological studies conducted on risk factors for ARV. Thus, the objective of

our study was to identify biosecurity and management practices associated with the presence of ARV among commercial broiler chicken flocks in Ontario.

MATERIALS AND METHODS

Sampling and Data Collection

A detailed description of our sampling method has been reported previously (Chapter 2). In brief, this study was part of a larger project designed to estimate the flock-level prevalence and identify risk factors for 13 pathogens of poultry health significance in the Ontario broiler industry. The sampling frame was all broiler producers in Ontario that were contracted with six major processing plants (five federal and one provincial). The requiredsample size was estimated to be 240 flocks (see chapter 2 for details). Approximately equal numbers of flocks were enrolled every 4 weeks during the July 2010 to January 2012 sampling periodto provide data on potential seasonal variation in pathogen prevalence. The number of flocks sampled at each plant every 4 weeks was proportional to the plant's market share of Ontario's broiler processing. The days on which each plant was visited were randomly-selected. For each sampling day, one flock was randomly-selected from the list of flocks scheduled to be slaughtered that day, and the corresponding producer was contacted and invited to participate in the study. At the processing plants, 15 blood samples, 15 cloacal swabs pooled into three samples (5 swabs per pool), and 15 whole intestines were conveniently collected from each flock. Samples were transported in coolers with ice packs to the Animal Health Laboratory, Guelph, Ontario. The samples were then further processed before submission for laboratory testing; one cecal tonsil was excised from each intestine using sterile instruments and pooled into three samples (5 tonsils per pool). Commercially available enzyme-linked immunosorbent assay (ELISA) kits (IDEXX Laboratories, Inc., Westbrook, Maine, USA) were used to detect ARV antibodies from serum

samples and each flock's ELISA mean titre was recorded from the laboratory report.Three pools of cecal tonsils, and 3 pools ofcloacal swabs were used for virus isolation in cell culture and then polymerase chain reaction (PCR) was carried out on cell culture fluids to detect viral shedding.

Data on biosecurity and management practices were collected for each flock; face-to-face interviews were conducted with the producers within a few days after shipment of the flock of interest.Face-to-face interviews were chosen over telephone or mail-in questionnaires because of their higher response rates (15). In addition to questionnaires, information was obtained from flock condemnation reports, On-Farm Food Safety Assurance Program records, flock management records, veterinary records (if applicable and with permission of the producer), and visitor log books to ensure comprehensive data collection for the flock. The information collected included variables related to 1) barn characteristics; 2) biosecurity; 3) pest control; 4) cleaning and disinfection of the barn and equipment; 5) water (source, types of treatment, and frequency of treatment); 6) bedding and litter condition; 7) feed; 8) bird environment, including temperature, air quality, and lighting; 9) manure management; and 10) bird supervision, including mortality and disease management. Questionnaires used to collect the information are shown in Appendix 2.

Data Analysis

The statistical analyses were performed using STATA Intercooled 10 (StataCorp, College Station, Texas, USA). Our data were modelled in two ways: 1) linear regression using the ELISA mean titre of the flock as the dependent variable; and 2) logistic regression using the PCR status of a sample (positive or negative for ARV) as the dependent variable and flock as a random intercept because there were six samples per flock (3 pooled cecal tonsil tissue samples and 3

pooled cloacal swab samples). Continuous and categorical explanatory variables used in the multivariable analyses are shown in Table 3.1.

Linear Regression Model

A univariable model was built for each independent variable to test its unconditional association with the dependent variable; all variables with $p \leq 0.2$on univariable screening, plus several variables of interest due to their biological plausibility (Table 3.2),regardless of their p-value on univariable screening, were considered for inclusion in a multivariable model. To avoid issues associated with collinearity, correlations between independent variables considered for inclusion in the multivariable model were assessed with Pearson (continuous variables) or Spearman (categorical variables) correlation coefficients; absolute values of > 0.8 were considered a concern for collinearity. If two variables were highly correlated, we excluded one of the variables (the one with the higher p-value on univariable screening). The relationship between the dependent variable and each continuous independent variable was assessed graphically using a lowess curve; if the linearity assumption was violated, we considered the addition of a quadratic term (if appropriate), transforming the continuous variable to achieve linearity, and categorizing the variable if the other procedures could not meet the modeling assumption. Categorization was achievedby setting cut-points based on graphical assessment of the distribution of the observations if a pattern was clear on visual inspection, otherwisequartileswere used.

The multivariable model was constructed using a manual backward elimination procedure. For categorical variables, a partial F-test was used to assess the overall significance of each variable; if a variable was not significant overall ($p > 0.05$) but at least one category was significant on a t-test, the variable was retained in the model. Each time a variable was removed

from the model, its confounding effect on other variables wasassessed; if the coefficient of another significant variable changed by more than 20%, the variable was deemed to have a confounding effect and it was retained in the model (even if it was not statistically significant) unless it was a potential intervening variable. For categorical variables, each category was contrasted with all other categories using the *lincom* command in STATA IC 10. All two-way interactions between significant variables in the final main effects model were tested. Only interactions that were statistically significant based on a partial F-test ($p \leq 0.05$) were left in the model.

We used a plot of the standardized residuals against the predicted values from the final multivariable linear model to assess homogeneity of variance,and a normal quantile plot to determine if the residuals were normally distributed. If the assumptions of homoscedasticity or normality were not met, a suitable transformation was selected (e.g., log transformation) and the model-building process was repeated.

Graphs were utilized to assess the fit of individual observations in the final multivariable model to identify outliers(observations with standardized residuals $\geq |3|$), observations with high leverage values, and influential observations (flocks with relatively large Cook's D and DFITS values).

Multi-level Logistic Regression Model

A univariable model was built for each independent variable to test its unconditional association with the dependent variable(the probability of the sample being positive for ARV on PCR) using flock as a random intercept.The distribution of the random effects was assumed to be Gaussian and the log likelihood was approximated by the adaptive quadrature method (16). All variables with $p \leq 0.2$on univariable screening, plus several variables of interest due to their

biological plausibility (regardless of their p-value on univariable screening), were considered for inclusion in a multivariable model. Assessment of collinearity between independent variables was similar to that described for the linear regression model. Lowess curves were used to assess the relationship between the log odds of the dependent variable and each continuous independent variable. The model-building process was identical to that described for the linear regression model, except that when removing a categorical variable, a likelihood ratio test was performed instead of a partialF-test. Visual assessment of a plot of the best linear unbiased predictors (BLUPS) was used to assess the normality of the flock-level residuals from the final model. The intra-class correlation coefficient (ICC) was estimated using the latent variable approach (15).

RESULTS

The farm-level ARV prevalence in our study population was 90.5% (Chapter 2). Flock/ farm-level data were available for 227 of 231 flocks; the characteristics of the study population have been described previously (Chapter 2). In brief, flock sizes ranged from 7,242 to 104,040 birds with a median of 25,092 birds (flock sizes were available for 200 flocks). The median weight of the birds at processing was 2.2 kg (range 1.7 to 3.1 kg) and the median age at shipment was 38 days (range 31 to 53 days). The mortality per flock ranged from 0.3 to 12.7% with a mean of 3.5% (95% CI: 3.3 to 3.7%) and a median of 3.2% (IQR: 2.5 to 4.3%). The number of PCR-positive samples per flock was 0 (111 flocks), 1 (53 flocks), 2 (29 flocks), 3 (16 flocks), 4 (9 flocks), 5 (10 flocks), and 6 (3 flocks). At the flock level, the ELISA mean titre ranged from 1 to 5,222 with a median of 519 (IQR: 259 to 1,052). Descriptive statistics for the continuous and categorical variables included in the multivariable analyses are shown in Table 3.1.

Multivariable Analysis

Linear Regression Model

A logarithmic transformation of the dependent variable was necessary to improve the fit of the model but robust standard errors were used since the graphical assessment of the standardized residuals indicated that the assumptions of homoscedasticity (Figure 3.1) and normality (Figure 3.2) were not met; therefore, we re-ran the final model using robust standard errors (Table 3.3). Collinearity was not detected between any of the variables considered for inclusion in the multivariable model. The variable age of flock at shipmentwas categorized using cut-points based on graphical assessment of the distribution of observations,whereas flock size was categorized using quartiles. The variables that were offered to the multivariable model are shown in Table 3.2. Variables that were significantly associated with the dependent variable at the univariable screening stage included age of flock at shipment, feed mill, flock exposure to CAV, flock exposure to IBDV, flock size, and season. Although the overall p-value for district was > 0.2, at least one category wassignificant. Variables that were not significant at the univariable screening stage yet were offered to the model due to their biological plausibility included barn disinfection, manure disposal on the farm, flock positive to ARV on PCR (at least one of six samples was positive to ARV on PCR), presence of other livestock on the farm, presence of other poultry on the farm, presence of pets on the farm, use of bootdip, and water source. Confounders were not identified during the model building process.

The final multivariable model includedtwo statistically significant variables (flock exposure to CAV, season), and one categorical variable (feed mill) in which at least one category was statistically significant. Four observations were identified as outliers (standardized residuals < -3) and three of those observations had a largeinfluence on the model (Cook's D > 0.06 and DFITS < -0.9); these flocks had the lowest ELISA mean titres (titres = 1 to 3). However, we did not find a valid reason to remove them from the model. Graphical assessment of the standardized

residuals indicated that the assumptions of homoscedasticity (Figure 3.1) and normality (Figure 3.2) were not met perfectly; therefore, we re-ran the final model using robust standard errors (Table 3.3). Co-infection with CAV was positively associated with the $\log_{10}$ELISA mean titre ($\beta = 0.62$, $p = 0.004$) (Table 3.3).

A statistically significant association was identified between the $\log_{10}$ ELISA mean titre and season (partial F-test $p = 0.027$); the $\log_{10}$ ELISA mean titre was significantly lower in the winter ($\beta = -0.67$, $p = 0.035$, 95% CI [-1.290, -0.047]) compared to autumn. The results of the contrasts also showed that the mean titre was significantly lower in the winter compared to the spring ($\beta = -0.95$, $p = 0.007$, 95% CI [-1.630, -0.267]) and summer ($\beta = -0.86$, $p = 0.004$, 95% CI [-1.452, -0.277]).

Although feed mill did not significantly contribute to explaining the overall variation in the $\log_{10}$ ELISA mean titre (partial F-test $p = 0.168$), the $\log_{10}$ ELISA mean titre was significantly lower in feed mill F compared to feed mills A ($\beta = -0.73$, $p = 0.026$,95% CI [-1.368, -0.089]), C ($\beta = -0.97$, $p = 0.009$,95% CI [-1.696, -0.242]), H ($\beta = -0.94$, $p = 0.014$,95% CI [-1.696, -0.191]), B ($\beta = -0.70$, $p = 0.068$,95% CI [-1.457, 0.051]), and E ($\beta = -0.75$, $p = 0.066$,95% CI [-1.558, 0.050]). Further, the mean titre was significantly lower in feed mill D compared to feed mill C ($\beta = -0.82$, $p = 0.042$,95% CI [-1.605, -0.030]). No significant interactions were identified. The R^2 of the final model was 0.149, indicating that the variables in the model (flock exposure to CAV, season, and feed mill) explained 14.9% of the variation in the $\log_{10}$ ELISA mean titre of the flock.

Multi-level Logistic Regression Model

The variables age of flock at shipment and ELISA mean titre were categorized using cut-points based on graphical assessment of the distribution of observations,whereas flock size was

categorized using quartiles. The variables that were offered to the multivariable model are shown in Table 3.4. Variables that were significantly associated with the dependent variable at the univariable screening stage included flock exposure to CAV, district, and ELISA mean titre of the flock. Variables that were not significant at the univariable screening stage yet were offered to the model due to their biological plausibility included age of flock at shipment, barn disinfection, flock exposure to IBDV, flock size, manure disposal on the farm,presence of other livestock on the farm, presence of other poultry on the farm, presence of pets on the farm, season, use of bootdip, and water source. Confounders were not identified during the model building process.

Although the final multivariable model was not significant overall (LRT $\chi^2 = 9.22$, $p = 0.056$),one statistically significant dichotomous variable (flock exposure to CAV), and one categorical variable (ELISA mean titre) in which at least one category was statistically significant, were identified(Table 3.5). Similar to the linear regression model, co-infection with CAV was positively associated with an increased risk of ARV infection (Table 3.5). Although the ELISA mean titre did not significantly contribute to explaining the overall variation in the PCR status of a sample ($p = 0.099$), the odds of a positive sample (compared to a negative sample) was significantly lower if the mean titre was 1 to 170 (OR = 0.43, $p = 0.030$) or 520 to 1,052 (OR = 0.38, $p = 0.018$) than if the mean titre was high (titre = 1,053 to 5,222). Statistically significant interactions were not identified. Visual assessment of a plot of the BLUPS indicated that the assumption of normality of the flock-level residuals was adequately met (Figure 3.3). The flock-level variance component (σ^2_{flock}) of the final model was 1.938. The ICCs of the intercept-only and final models were $\rho = 0.385$ and $\rho = 0.371$, respectively. The ICC for the full model indicates that 37% of the variation in the PCR status of a sample was between flocks. The

similarity in the ICC values of the null and final models indicates that the variables remaining in the final model (flock exposure to CAV and ELISA mean ARV titre) did not explain much of the between-flock variation in the PCR status of a sample.

DISCUSSION

Our objective was to identify biosecurity and management practices associated with the presence of ARV among commercial broiler chicken flocks in Ontario. Our approach was to analyze the data in two ways. First, to use the ELISA mean antibody titre of the flock (evidence ofan immune response to exposure) as the dependent variable, and second, to use the PCR status of a sample (evidence of viral shedding) as the dependent variable. Both models revealed that exposure to CAV was a risk factor for ARV. McNeilly et al. (1995) found that chicks inoculated with both CAV and ARV showed significantly lower weight gain compared to chicks inoculated with CAV alone (8). The researchers also noted that synergism between certain strains of ARV and CAV might have enhanced the effect of ARV pathogenesis (8).

Our previous analysis of the seasonal variation of ARV prevalence among Ontario broiler chicken flocks showed that there were significantly lower odds of ARV presence in the summer compared to the other seasons when a combination of ELISA mean titre and the PCR status of the flock were used as a dependent variable, and when exposure to CAV and feed mill were not accounted for (Chapter 2). The farm-level prevalence of ARV was very high across all seasons, ranging from 72.2% in the summer to 100% in the spring, autumn, and winter (Chapter 2). In our linear regression model, flocks raised in the winter had significantly lower mean titres when compared to flocks raised in the spring, summer, or autumn, after controlling for the CAV exposure status of the flock and the feed mill where feed for the flock was sourced. The prevalence of CAV in our study population was 77.1% (17), ranging from 69.6% in the summer

to 85.4% in the winter (18). Although season alone did not significantly explain the overall variation in the prevalence of CAV exposure in our study population, there was a trend toward exposure to CAV being lower in the summer compared to the winter (18). The lack of a significant interaction between season and CAV in our linear regression model, and the different seasonal distributions of ARV and CAV in our study population, suggest that the effect of season on ARV exposure does not depend on exposure to CAV.

Although feed mill did not significantly contribute to explaining the overall variation in the flock ARV mean titre, we identified two feed mills that had significantly lower mean titres than one or more other feed mills, controlling for flock exposure to CAV and season. Of interest, differences between feed mills were also identified with respect to the risk of flock exposure to CAV in our study population (19). The reason for our finding is unclear. Possible reasons might include aspects of feed mill location, feed ingredients, feed mixing or pelleting procedures, pest control, or cleaning and disinfection of equipment and delivery trucks or biosecurity measures followed on farm during delivery. Further research is required to understand the role of feed mills on the risk of ARV and other pathogens in broiler flocks in Ontario.

In our multi-level logistic regression model, the odds of a positive PCR sample were generally 2.0 to 2.6 times higherwhen the flock ELISA mean titre was high (1,053 – 5,222) than when the flock mean titre was very low (1 to 170), low (171 to 519), or moderate (520 to 1,052), controlling for the CAV exposure status of the flock and clustering of samples at the flock level. This finding suggests that, at the time of processing, flocks with high titres were more likely to be shedding the virus than flocks with lower titres.

Modeling our data in two different ways revealed an interesting relationship between the ELISA mean titre and the PCR test results. In the linear model, the PCR status of a flock was not

significantly associated with the $\log_{10}$ ELISA mean titre, whereas in the logistic model, the ELISA mean titre was significantly associated with the PCR status of a sample, controlling for clustering of samples at the flock level. In the linear model, a flock was considered PCR-positive if at least 1 of 6 samples tested positive. Of the 120 flocks that tested positive to PCR in our study, 44% had only one positive sample; the other 56% of the PCR-positive flocks had two or more positive samples. The number of PCR-positive samples was not accounted for in the linear model. Thus, the difference between model results might be due to the difference between the outcomes as well as the power of each model; the dependent variable of the linear model was measure at a flock level, whereas the dependent variable of the logistic model was measure at a sample level, therefore the model may have had more power compared to the linear model.

The high prevalence of ARV in our study population likely limited our ability to identify biosecurity and management practices associated with the presence of ARV. However, several variables (age of flock at shipment, and flock exposure to IBDV) were identified as being associated with either an immune response to ARV exposure, or viral shedding of ARV, at the univariable screening stage.Therefore, future research may benefit from exploring these variables further.One additional limitation that could explain the results of our analyses is that we did not distinguish ARV genotypes.It is generally recognized that approximately 80% of isolated ARV strains are non-pathogenic (1). This resulted in low specificity of outcome, which in turn might have resulted in non-differentiated misclassification bias. In addition, the lack of distinction likely made it difficult to evaluate ARV presence and its association with the investigated variables. Therefore, future research may benefit from genotyping isolates and identifying biosecurity and management practices associated with pathogenic genotypes.

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Table 3.1. Descriptive statistics of variables of interest in a study assessing risk factors for avian reovirus (ARV) among commercial broiler chicken flocks sampled at processing between July 2010 and January 2012 in Ontario, Canada

Dependent Variables		
Linear regression: $log_{10}$of the ELISA mean titre of the flock (n = 231)[a]		
Multi-level logistic regression: PCR status of sample (n = 1,386 samples from 231 flocks)[b]		
Independent Variables		**Description**
Continuous		**Median (range)**
	Age of flock at shipment (days; n = 223)	38 (31 to 53)
	ELISA mean titre of flock (n = 231)	519 (1 to 5,222)
	Flock size (n = 200)	25,092 (7,242 to 104,040)
Categorical		**Category (percentage of flocks)**
	Age of flock at shipment (days; n = 223)[c]	31 to 36 (12.6) 37 to 38 (28.2) 39 to 40 (26.0) 41 to 53 (33.2)
	District (n = 231)	1 (9.1) 2 (16.5) 3 (19.5) 4 (7.8) 5 (6.5) 6 (4.3) 7 (12.6) 8 (14.2) 9 (9.5)
	ELISA mean titre of flock (n = 231)[a]	1 to 170 (14.7) 171 to 519 (35.5) 520 to 1,052 (25.5) 1,053 to 5,222 (24.3)
	Feedmill (n = 223)[d]	A (24.7) B (10.3) C (7.6) D (8.5) E (5.8) F (9.9) G (18.8) H (8.6) I (5.8)
	Flock exposed to chicken anemia virus (n = 231)	Yes (77.1) No (22.9)
	Flock exposed to infectious bursal disease virus (n = 227)	Yes (57.6) No (40.7)

	Flock positive to ARV on PCR (n = 231)[b]	Yes (51.9) No (48.1)
	Flock size (n = 200)[e]	0 to 18,162 (27.5) 18,163 to 24,366 (28.0) 24,367 to 34,060 (27.5) 34,061 to 104,040 (17.0)
	Manure disposal on farm (n = 224)	Yes (80.8) No (19.2)
	Presence of other livestock on the farm (n = 221)	Yes (41.2) No (58.8)
	Season (n = 229)	Spring (12.7) Summer (34.1) Autumn (36.2) Winter (17.0)

[a] Enzyme-linked immunosorbent assay (ELISA) results were used as the dependent variable in the linear regression model, and an independent variable (categorized by setting cut-points based on graphical assessment of the distribution of the observations) in the logistic regression model.
[b] Polymerase chain reaction (PCR) results were used as the dependent variable in the multi-level logistic regression model with flock as a random intercept because six samples were collected per flock, and an independent variable (dichotomized as PCR-positive or negative at the flock level) in the linear regression model.
[c]Categorization of the continuous variable was achievedby setting cut-points based on graphical assessment of the distribution of the observations.
[d]This variable was only included in the linear regression model.
[e]Categorization of the continuous variable was achievedusing quartiles.

Table 3.2. Univariable linear regression models for factors associated with avian reovirus (ARV)[a] in commercial broiler chicken flocks in Ontario (n = 227 flocks)

Variable	Coefficient	95% CI of coefficient	p-value (t-test)	p-value (partial F-test)
Age of flock at shipment				0.029
31 to 36	Referent			
37 to 38	-0.14	(-0.69, 0.41)	0.626	
39 to 40	0.05	(-0.51, 0.60)	0.867	
41 to 53	0.47	(-0.07, 1.00)	0.089	
Barn disinfection[b]				
Yes	-0.008	(-0.37, 0.35)	0.963	
No	Referent			
District[c]				0.467
1	-0.53	(-1.22, 0.17)	0.136	
2	-0.41	(-1.00, 0.18)	0.172	
3	-0.41	(-0.98, 0.17)	0.163	
4	0.09	(-0.65, 0.83)	0.805	
5	-0.47	(-1.24, 0.30)	0.232	
6	-0.25	(-1.18, 0.68)	0.603	
7	-0.15	(-0.78, 0.48)	0.634	
8	Referent			
9	-0.71	(-1.40, -0.01)	0.046	
Feed mill				0.114
A	Referent			
B	0.04	(-0.57, 0.65)	0.890	
C	0.47	(-0.21, 1.15)	0.176	
D	-0.42	(-1.07, 0.24)	0.210	
E	-0.01	(-0.79, 0.78)	0.990	
F	-0.66	(-1.29, -0.05)	0.036	
G	0.01	(-0.50, 0.51)	0.983	
H	0.34	(-0.31, 0.99)	0.306	
I	-0.40	(-1.16, 0.36)	0.299	
Flock exposed to chicken anemia virus				
Yes	0.59	(0.22, 0.97)	0.002	
No	Referent			
Flock exposed to infectious bursal disease virus				
Yes	0.33	(0, 0.65)	0.050	
No	Referent			

Variable	Coefficient	95% CI of coefficient	p-value (t-test)	p-value (partial F-test)
Flock size				0.088
7,242 to 18,162	Referent			
18,163 to 24,366	0.32	(-0.23, 0.87)	0.254	
24,367 to 34,060	-0.23	(-0.81, 0.36)	0.441	
34,061 to 104,040	0.11	(-0.47, 0.69)	0.705	
Manure disposal on the farm[b]				
Yes	0.22	(-0.20, 0.63)	0.303	
No	Referent			
Flock positive to ARV on PCR[b, d]				
Yes	-0.15	(-0.48, 0.18)	0.365	
No	Referent			
Presence of other livestock on the farm[b]				
Yes	-0.02	(-0.35, 0.32)	0.927	
No	Referent			
Presence of other poultry on the farm[b]				
Yes	0.02	(-0.62, 0.67)	0.943	
No	Referent			
Presence of pets on the farm[b]				
Yes	0.02	(-0.36, 0.41)	0.905	
No	Referent			
Season				0.023
Spring	0.24	(-0.28,0.76)	0.359	
Summer	0.21	(-0.17,0.59)	0.278	
Autumn	Referent			
Winter	-0.50	(-0.97, -0.03)	0.038	
Use of bootdip[b]				
Yes	-0.63	(-1.87, 0.62)	0.323	
No	Referent			
Water source[b]				
Municipal	Referent			
Others	0.01	(-0.45, 0.46)	0.980	

[a] The dependent variable was the $\log_{10}$ ELISA mean titre of flock

[b] The variable wasnot significant at the univariable screening stage yet was offered to the multivariable model due to its biological plausibility.

[c] Although the overall p-value was > 0.2 at the univariable screening stage, at least one category wassignificant; therefore, the variable was offered to the multivariable model.

[d] At least one of six samples was positive to ARV on polymerase chain reaction (PCR).

Table 3.3. Final multivariable linear regression model using robust standard errors examining associations between factors associated with avian reovirus (ARV)[a] in commercial broiler chicken flocks in Ontario (n = 221 flocks)

Variable	Coefficient	95% CI of coefficient	p-value (t-test)	p-value (partial F-test)
Feed mill				0.168
A	Referent			
B	-0.03	(-0.59, 0.54)	0.928	
C	0.24	(-0.32, 0.80)	0.400	
D	-0.58	(-1.29, 0.13)	0.110	
E	0.03	(-0.61, 0.66)	0.938	
F	-0.73	(-1.37, -0.09)	0.026	
G	-0.23	-(0.70, 0.24)	0.337	
H	0.21	(-0.37, 0.80)	0.473	
I	-0.31	(-1.22, 0.60)	0.500	
Flock exposed to chicken anemia virus				
Yes	0.62	(0.20,1.04)	0.004	
No	Referent			
Season				0.027
Spring	0.28	(-0.16, 0.72	0.216	
Summer	0.20	(-0.15, 0.54)	0.266	
Autumn	Referent			
Winter	-0.67	(-1.30, -0.05)	0.035	
intercept	5.79	(5.31, 6.27)	0.001	

[a] The dependent variable was the $\log_{10}$ ELISA mean titre of flock. Model summary: $p < 0.017$; $R^2 = 0.149$

Table 3.4. Univariable logistic regression model with flock as a random interceptexamining associations between factors associated with avian reovirus (ARV)[a] in commercial broiler chicken flocks in Ontario (n = 227 flocks)

Variable	Odds ratio	Coefficient	95% CI of coefficient	p-value (Wald's test)	p-value (Likelihood ratio test)
Age of flock at shipment[b]					0.584
31 to 36		Referent			
37 to 38	1.85	0.616	(-0.27, 1.50)	0.174	
39 to 40	1.69	0.524	(-0.37, 1.42)	0.253	
41 to 53	1.70	0.530	(-0.34, 1.40)	0.233	
Barn disinfection[b]					
Yes	1.04	0.04	(-0.51, 0.59)	0.878	
No		Referent			
Flock exposed to chicken anemia virus					
Yes	1.73	0.55	(-0.07, 1.16)	0.080	
No		Referent			
Flock exposed to infectious bursal disease virus[b]					
Yes	1.17	0.16	(-0.35, 0.67)	0.543	
No		Referent			
Flock size[b]					0.629
7,242 to 18,162		Referent			
18,163 to 24,366	1.04	0.04	(-0.70, 0.77)	0.922	
24,367 to 34,060	1.04	0.19	(-0.54, 0.91)	0.616	
34,061 to 104,040	1.65	0.50	(-0.31, 1.31)	0.227	
District					0.178
1	1.90	0.64	(0.37, 1.65)	0.213	
2	0.71	-0.34	(1.25, 0.56)	0.458	
3	1.74	0.55	(-0.29, 1.40)	0.203	
4	0.72	-0.33	(-1.49, 0.82)	0.571	
5	2.52	0.92	(-0.18, 2.03)	0.101	
6	0.67	-0.39	(-1.86, 1.06)	0.592	
7	0.97	-0.03	(-0.99, 0.92)	0.942	
8		Referent			
9	0.91	-0.09	(-1.14, 0.94)	0.853	

Variable	Odds ratio	Coefficient	95% CI of coefficient	p-value (Wald's test)	p-value (Likelihood ratio test)
Feed mill					0.438
A		Referent			
B	0.89	-0.12	(-1.82, 1.58)	0.892	
C	0.24	-1.42	(-3.86, 1.02)	0.255	
D	0.90	-0.11	(-1.93, 1.72)	0.909	
E	2.72	1.0	(-0.80, 2.79)	0.275	
F	0.70	-0.36	(-2.08, 1.36)	0.685	
G	0.59	-0.52	(-2.02, 0.98)	0.496	
H	0.10	-2.31	(-5.08, 0.47)	0.103	
I	0.77	-0.26	(-2.38, 1.85)	0.807	
ELISA mean titre of flock					0.172
1 to 170	0.46	-0.78	(-1.54, -0.02)	0.045	
171 to 519	0.55	-0.59	(-1.39, 0.21)	0.145	
520 to 1,052	0.43	-0.84	(-1.65, -0.04)	0.041	
1,053 to 5,222		Referent			
Manure disposal on the farm[b]					
Yes	1.35	0.30	(-0.35, 0.96)	0.363	
No		Referent			
Presence of other livestock on the farm[b]					
Yes	1.29	0.28	(-0.22, 0.79)	0.269	
No		Referent			
Presence of other poultry on the farm[b]					
Yes	1.11	0.11	(-0.84, 1.05)	0.825	
No		Referent			
Presence of pets on the farm[b]					
Yes	1.01	0.013	(-0.58, 0.61)	0.965	
No		Referent			
Season[b]					0.328
Spring	0.71	-0.34	(-1.14, 0.46)	0.406	
Summer	0.58	-0.54	(-1.12, 0.05)	0.071	
Autumn		Referent			
Winter	0.68	-0.38	(-1.10, 0.33)	0.292	
Use of bootdip[b]					
Yes	1.14	0.14	(-1.77, 2.05)	0.888	
No		Referent			
Water source[b]					
Municipal		Referent			
Other	1.02	0.02	(-1.36, 1.39)	0.980	

[a] The dependent variable was the PCR status of a sample (positive or negative for ARV). Flock was included as a random intercept because there were six samples per flock (3 pooled cecal tonsil tissue samples and 3 pooled cloacal swab samples).
[b] The variable wasnot significant at the univariable screening stage yet was offered to the multivariable model due to its biological plausibility.

Table 3.5. Final multivariable logistic regression model with flock as a random intercept for factors associated with avian reovirus (ARV)[a] in commercial broiler chicken flocks in Ontario (n = 227 flocks)

Variable	Odds ratio	Coefficient	95%CI of coefficient	p-value (Wald's test)	p-value (Likelihood ratio test)
Flock exposed to chicken anemia virus					
Yes	1.92	0.65	(0.04, 1.27)	0.038	
No		Referent			
ELISA mean titre of flock					0.099
1 to 170	0.43	-0.84	(-1.59, -0.08)	0.030	
171 to 519	0.50	-0.69	(-1.49, 0.10)	0.088	
520 to 1,052	0.38	-0.97	(-1.79, -0.17)	0.018	
1,053 to 5,222		Referent			
intercept		-1.62	(-2.37, -0.87)	0.001	

[a] The dependent variable was the PCR status of a sample (positive or negative for ARV). Flock was included as a random intercept because there were six samples per flock (3 pooled cecal tonsil tissue samples and 3 pooled cloacal swab samples). Model summary: overall p-value = 0.056; random effects parameter [standard deviation = 1.392; 95% CI (1.134, 1.708)]

Figure 3.1. Plot of the standardized residuals against the predicted values from the multivariable linear regression model to assess the assumption of homogeneity of variance

Figure 3.2. Normal quantile plot of the standardized residuals from the multivariable linear regression model to evaluate the assumption of normality of residuals

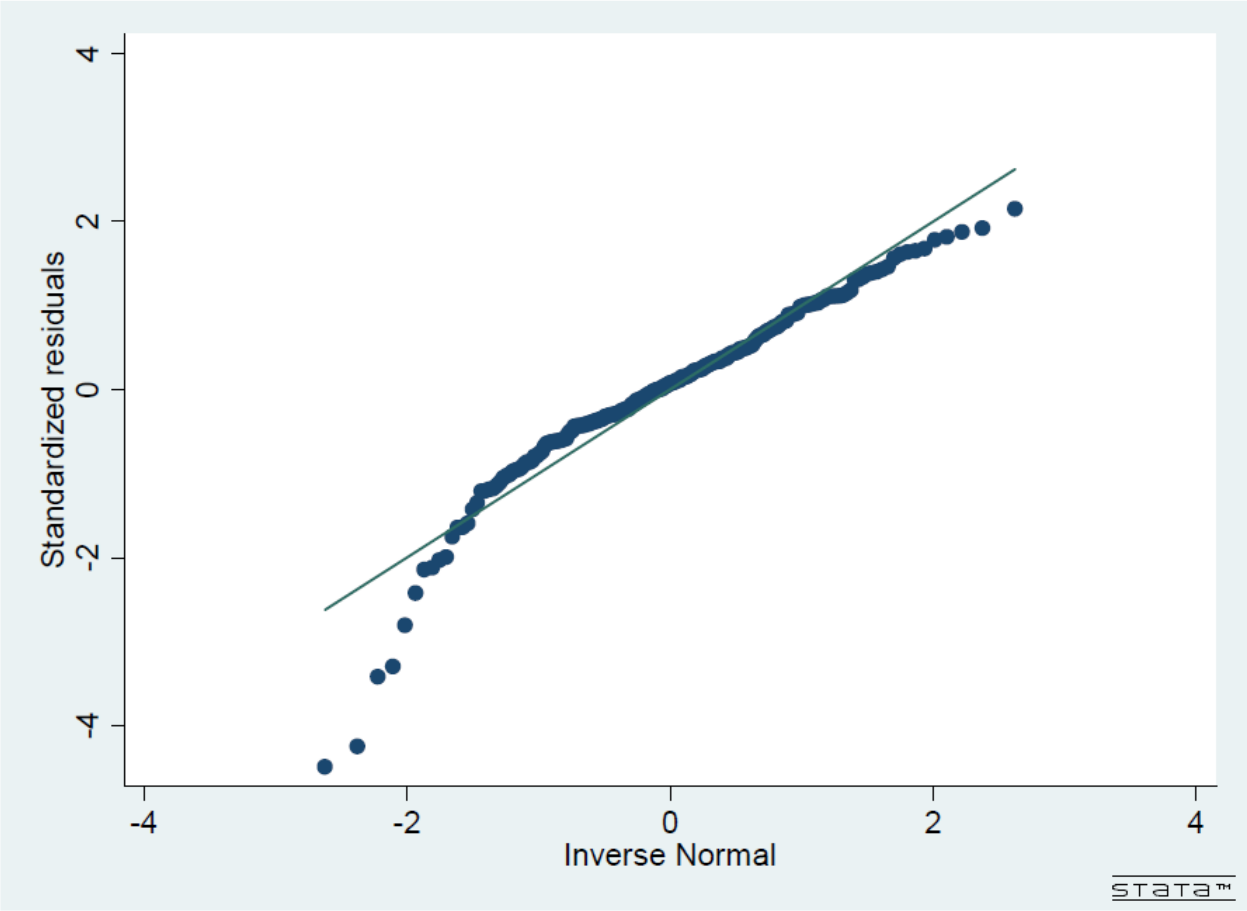
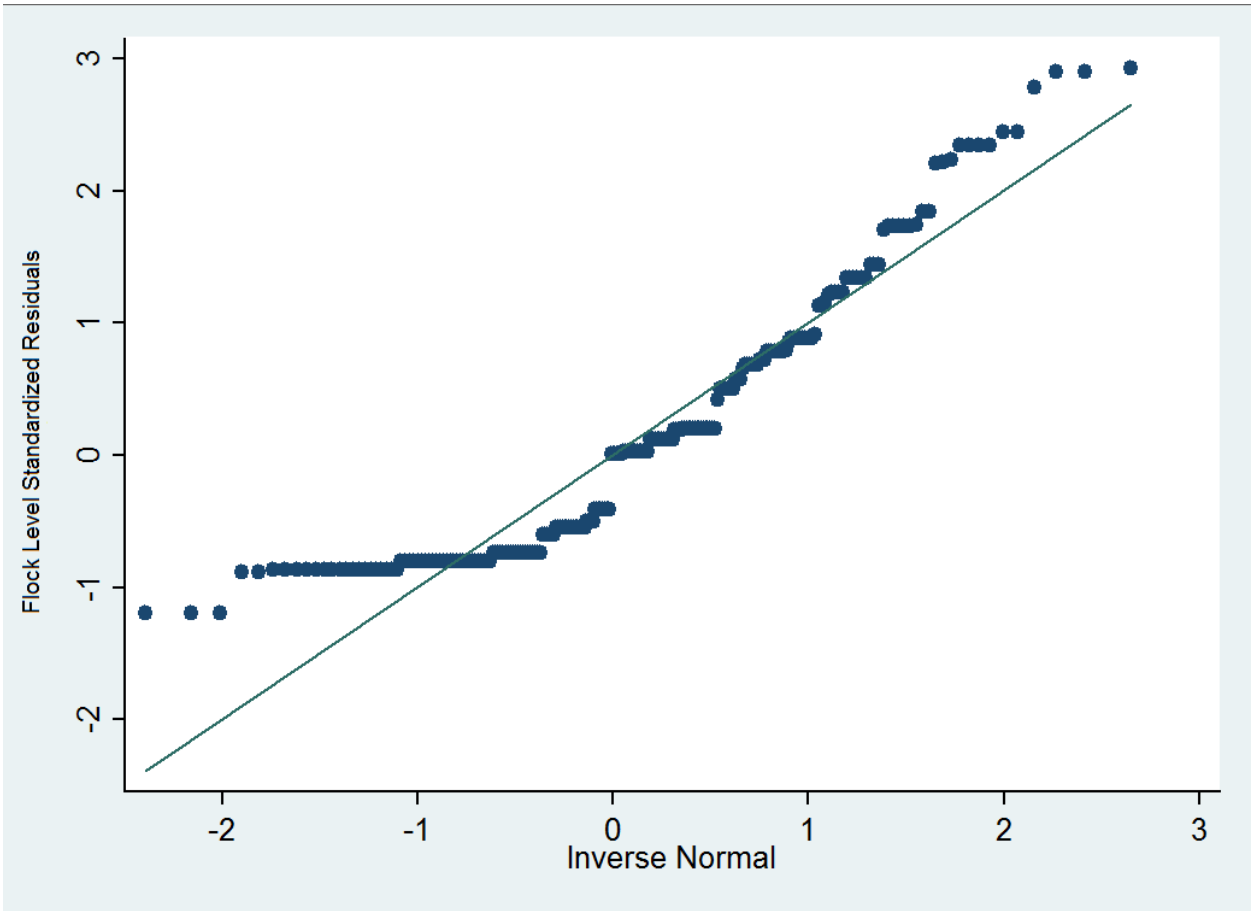


Figure 3.3. Normal quantile plot of best linear unbiased predictorsfrom the multivariable logistic regression model to evaluate normality



CHAPTER 4: CONCLUSIONS

Avian reoviruses (ARVs) are associated with a variety of diseases in chickens, including respiratory and enteric disease, hydropericardium, pericarditis, myocarditis, hepatitis, and tenosynovitis (1). Affected birds are either culled during the grow-out period or condemned at processing. This is a serious economic concern in the broiler industry due to costs associated with feed, labour, and lost production.

A review of the scientific literature shows that ARV is endemic in the broiler industry around the globe, as the reported seroprevalence of ARV ranges from 41.0 to 98.3% (2-4). Both horizontal and vertical transmission of the virus are possible (5-8). Common horizontal modes of ARV include exposure through broken skin of the foot pad or the respiratory route (1).The traditional approach to control ARV infection in broilers has been vaccination of parent flocks and strict biosecurity/ management practices. Factors that have been linked to outcome of ARV infection include host age, host immune status, strains of ARV, and co-infection with other pathogens (1).

Despite the experimental evidence on ARV transmission, the epidemiology of ARV is not fully understood, and there was insufficient information on the prevalence or distribution of ARV among Ontario broiler chicken flocks.This knowledge gap formed the basis of the objectives of the thesis, which were: 1) to establish the baseline prevalence, geographical distribution, and seasonal variation of ARV, and 2) to identify any management and biosecurity associated risk factors on ARV infection among commercial broiler chicken flocks in Ontario.

Prevalence of ARV

To our knowledge, this study was the first epidemiological study, since 1979, to investigate the prevalence and distribution of the pathogen in the commercial broiler population in Canada. A representative sample of 231 Ontario broiler chicken flocks were included in the study, and biological samples, which included blood, cloacal swabs, and caecal tonsils, were collected at processing. The total number of samples tested per flock using polymerase chain reaction (PCR) was 6 (3 pooled samples of caecal tonsil tissues and 3 pooled samples of cloacal swabs); if ≥ 1 of 6 samples tested positive, the flock was considered to be PCR-positive). The flock's mean enzyme-linked immunosorbent assay (ELISA) titre andPCR status were used to determine a flock's ARV exposure status. Thefindings revealed that ARV infection was common (90.5%) among broiler flocks in Ontario; however, this was not synonymous with the ARV-associated diseases. The ARV prevalence in Ontario was higher than the reported ARV prevalence in Nigeria (41.0%) (2), and similar to the reported prevalence in Iran (98.3%) (3) and Turkey (70.6 to 77.2%) (4), despite differences between studies, including the methods used to classify flocks (positive/negative), the age of broilers at sampling, antibody titre thresholds for ARV seropositivity, and vaccination of parent flocks. Avian reovirusis active at temperatures up to 50°C (9), and is able to survive for up to 10 days on feathers, wood shavings, glass, rubber, and galvanised metal, and for 10 weeks in water (10). The virus can survive under farm conditions for 12 to 15 weeks (10). It is heat resistant, stable in a wide spectrum of pH (3.0 to 9.0), and resistant to disinfectants commonly used in poultry houses (9). The hardiness and survivability of the virus likely explains the high prevalence in Ontario and other poultry-producing countries.

Factors associated with ARV

District

The results of a univariable logistic regression model examining the association between ARV presence and broiler district (Chapter 2) showed that districts 1, 3, 4, and 5 (prevalence of 80 to 86%) had a significantly lower prevalence of ARV than districts 2 and 8 (96 to 100%). Despite these statistical differences, the prevalence was very high across the province, ranging from 80 to 100%. The minor variation in the geographical distribution of ARV might be attributed to factors that differ between districts, such as the density of farms, environmental challenges, or subtle differences in management and biosecurity practices.

Season

To our knowledge, this study was the first to investigate the seasonal variation in farm-level ARV prevalence. The ARV prevalence was very high in all seasons (spring: 100%, summer: 72.2%, autumn: 100%, winter: 100%). The Chicken Farmers of Ontario's On-Farm Food Safety Assurance Program dictates that broiler chicken barns must be cleaned and disinfected at least once per year. It is possible that many broiler producers disinfect the barns in the summer when the temperature and weather are more favourable for the task, which could explain the lower ARV farm-level prevalence in the summer compared to other seasons. The results from the univariable exact logistic regression model showed that relative to summer, the other seasons were at significantly greater odds of ARV presencewhen a combination of ELISA mean titre and the PCR status of the flock were used as a dependent variable,and when exposure to CAV and feed mill were not accounted for (Chapter 2). However, in the linear regression model, flocks raised in the winter had significantly lower mean titres when compared to flocks raised in the spring, summer, or autumn, controlling for the CAV exposure status of the flock and the feed mill where feed for the flock was sourced (Chapter 3). The overall farm-level

prevalence of CAV in our study population was 77.1% (11), ranging from 69.6% in the summer to 85.4% in the winter (12). Although season alone did not significantly explain the overall variation in the prevalence of CAV exposure in our study population, there was a trend toward exposure to CAV being higher in the winter compared to the summer (12). The lack of a significant interaction between season and CAV in our linear regression model, and the different seasonal distributions of ARV and CAV in our study population, suggest that the effect of CAV is independent of season and implies that there are other, unmeasured seasonal influences contributing to ARV exposure.

Farm/flock-level risk factors

Two farm/flock-level risk factors for ARV were identified (Chapter 3). The first of these was flock exposure to CAV, which was associated with an increased risk of ARV. This association was found using two different modeling approaches. McNeilly et al. (1995) found that chicks inoculated with both CAV and ARV showed significantly lower weight gain compared to chicks inoculated with CAV alone (13). The researchers also noted that synergism between certain strains of ARV and CAV might have enhanced the effect of ARV pathogenesis (13).

The second farm/flock-level risk factor that we identified was the feed mill that supplied feed to the study flock. Although feed mill did not significantly contribute to explaining the overall variation in the flock ARV mean titre, two feed mills had significantly lower mean titres than one or more other feed mills, controlling for flock exposure to CAV and season. Of interest, differences between feed mills were also identified with respect to the risk of flock exposure to CAV in our study population (14). The reason for this finding is unclear. Possible reasons might include aspects of feed mill location, feed ingredients, feed mixing or pelleting procedures, pest

control, cleaning and disinfection of equipment and delivery trucks, or biosecurity measures followed on farm during delivery. Further research is required to understand the role of feed mills on the risk of ARV and other pathogens in broiler flocks in Ontario.

Relationship between flock ELISA mean titre and PCR results

A flock was considered to be PCR-positive if one or more samples tested positive on PCR. In the study population, 51.9% of the flocks were PCR-positive and 48.1% were PCR-negative (Chapter 2). Further, the distribution of ELISA mean titres was similar between the PCR-positive and PCR-negative flocks. The flock's mean ELISA titre and PCR status were used to determine its ARV exposure status. Modeling the data in two different ways revealed an interesting relationship between the ELISA mean titre and the PCR test results. In the linear model, the PCR status of a flock was not significantly associated with the $\log_{10}$ ELISA mean titre, whereas in the logistic model, the ELISA mean titre was significantly associated with the PCR status of a sample, controlling for clustering of samples at the flock level. Of the 120 flocks that tested positive to PCR in this study, 44% had only one positive sample; the other 56% of the PCR-positive flocks had two or more positive samples. The number of PCR-positive samples was not accounted for in the linear model. Thus, the difference between model results might be due to the difference between the outcomes and number of observations of each model; the dependent variable of the linear model was measured at a flock level, whereas the dependent variable of the logistic model was measured at a sample level, with the latter model having more power to detect associations.

Relationship between flock mortality and flock ELISA mean titre and PCR results

The mean mortality per flock in our study population was 3.5%, which is consistent with the provincial average flock mortality in Ontario between 2005 and 2009 (4.0%; Ouckama, personal communication, 2013).Flock mortality wasnot significantly associated with flock ELISA mean titreorflock PCR status (positive vs. negative) suggesting that there was no direct relationship between mortality and antibody titres or viral shedding in this baseline surveillance study. However, given that natural mortality and culling could not be differentiated, themortality data might be a surrogate for poor management or heavy culling for ARV-associated diseases or other diseases. Additionally, the non-significant association between mortality and antibody titres and PCR status might be related to a lack of differentiation between pathogenic and non-pathogenic strains (i.e. we did not genoype the isolates).It is generally recognized that approximately 80% of isolated ARV strains are non-pathogenic (1).

Strengths and Weaknesses

This thesis was a part of a larger project (the Enhanced Surveillance Project [ESP]) aimed at determining the flock-level prevalence, and biosecurity and management practices associated with 13 pathogens of poultry health significance among commercial Ontario broiler chicken flocks. The design of the ESP ensured a representative sample of Ontario's broiler population in the following ways. Approximately equal numbers of flocks were enrolled every 4 weeks during the study periodto account for potential seasonal variation in pathogen prevalence. The samples were collected from six processing plants that represent approximately 70% of broiler chickens processed in the province, and the number of flocks sampled at each plant was proportional to the plant's market share of Ontario's broiler processing.Statistical software was used to randomly allocate specific sampling days to each plant, and numbered coins were used to

randomly select one flock from the list of flocks scheduled to be slaughtered on each sampling day. To ensure that samples were representative of the flock, approximately equal numbers of samples from each truckload of birds were collected. The methods used to select flocks resulted in an excellent representation of the distribution of the province's broiler production (12).

The study results may have been affected by the following factors: 1) there was little variability for some of the variables due to standard practices (e.g. CFO's On-Farm Food Safety Assurance Program); and 2) the choice of the cut-point used in the classification of flocks as positive or negative for ARV. For ELISA mean titres, the cut-point(396) specified in the IDEXX reference guide was used as a component of the criteria to classify flocks as positive or negative for ARV. Thus, our testing methodfor antibody response resulted in low specificity of outcome. In turn, this might have resulted in an over-estimation of ARV prevalence.

Recommendations

One limitation of this study is that the ARV isolates were notdistinguished. Avian reovirus has been associated with several poultry diseases, and yet the pathogenesis is unclear and its presentation is likely influenced by other factors. Approximately 80% of isolated ARV strains are non-pathogenic (1). At the present time, commercial ELISA cannot distinguish between pathogenic and non-pathogenic strains of ARV; therefore, it is not known what percentage of flocks in the study was exposed to pathogenic strains. Although it was possible to identify associations between ARV and potential risk factors, it was not possible to identify risk factors for pathogenic strains. It is therefore recommended that future studies include genotyping in order to better understand the epidemiology of ARV. Such knowledge will facilitate the development of effective disease prevention and control strategies, and mitigate the economic impact of the virus.

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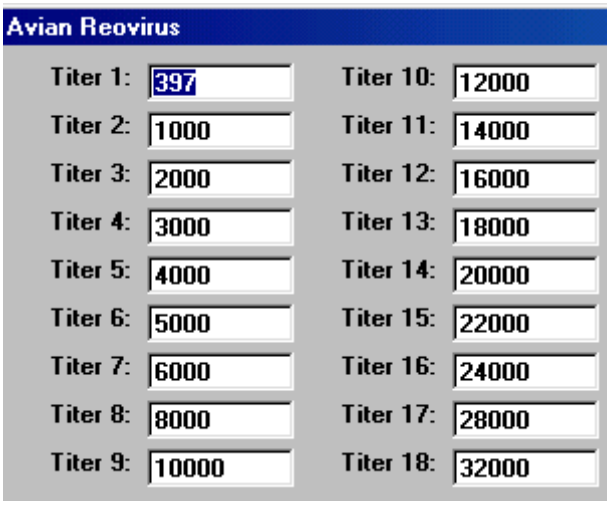
APPENDICES

Appendix 1.

Titre groups provided by IDEXX (IDEXX Laboratories, Inc., Westbrook, Maine, USA) for their enzyme-linked immunosorbent assay (ELISA).



Appendix 2.
Questionnaires used to collect data on biosecurity and flock management practices during face-to-face interview.

Questionnaire: Enhanced Surveillance for viral and bacterial pathogens in Ontario broilers

Abbreviations: **FS** = Flock-specific management **GM** = General Management **N/A** = Not Applicable

PART A

NAME OF PERSON INTERVIEWED: _____ POSITION: _____

FS: Quota Period: _____ (e.g. A-98)

GENERAL INFORMATION

Briefly describe your operation in terms of the number of farms (F), number of barns (B), and number of employees, including owner or manager (E),includingall-in-all-out practices (AIAO)?

_____

_____

What is your current crop quota cycle? _____ 8-week (6 to 7 flocks/year)
_____ 9-week (5 to 6 flocks/year)
_____10-week (5 to 6 flocks/year)
_____ 12-week (4 to 5 flocks/year)

What is your annual production? _____ birds/year or _____ kg/year

BARN CHARACTERISTICS

FS: For the flock that was slaughtered on _____, did the flock consist of birds from more than one barn?

_____ Yes (*go to Part C*)
_____ No

If yes, how many other barns?_____

If yes, were the barns adjoining (e.g. shared entrance) _____ Yes
_____ No

FS: Barn construction where flock was housed (**BARN 1**) (*Check all that apply*):

Barn Level	**Floor Area (m^2 or ft^2)**	**Floor Material**	**Wall Material (inside barn)**	**Ceiling Material**
Lower	_____	_____ Wood 1 – solid or plywood _____ Wood 2 – particle or pressed board _____ Concrete _____ Other	_____ Wood 1 – solid or plywood _____ Wood 2 – particle or pressed board _____ Concrete only _____ Concrete at bottom (height _____) & wood 1 at top _____ Concrete at bottom (height _____) & wood 2 at top _____ Plastic only _____ Metal only _____ Other	_____ Wood 1 – solid or plywood _____ Wood 2 – particle or pressed board _____ Concrete _____ Plastic _____ Metal _____ Other
Middle	_____ or _____ N/A	_____ Wood 1 – solid or plywood _____ Wood 2 – particle or pressed board _____ Concrete _____ Other	_____ Wood 1 – solid or plywood _____ Wood 2 – particle or pressed board _____ Concrete only _____ Concrete at bottom (height _____) & wood 1 at top _____ Concrete at bottom (height _____) & wood 2 at top _____ Plastic only _____ Metal only _____ Other	_____ Wood 1 – solid or plywood _____ Wood 2 – particle or pressed board _____ Concrete _____ Plastic _____ Metal _____ Other

Barn Level	Floor Area (m^2 or ft^2)	Floor Material	Wall Material (inside barn)	Ceiling Material
Upper	_____ or _____ N/A	_____ Wood 1 – solid or plywood _____ Wood 2 – particle or pressed board _____ Concrete _____ Other	_____ Wood 1 – solid or plywood _____ Wood 2 – particle or pressed board _____ Concrete only _____ Concrete at bottom (height _____) & wood 1 at top _____ Concrete at bottom (height _____) & wood 2 at top _____ Plastic only _____ Metal only _____ Other	_____ Wood 1 – solid or plywood _____ Wood 2 – particle or pressed board _____ Concrete _____ Plastic _____ Metal _____ Other

FS: What type of ventilation does Barn 1 have?
_____ Cross ventilation
_____ Tunnel ventilation
_____ Double-sided ventilation
_____ Other (_____)

FS: How wide is barn 1? _____ m or _____ ft

FS: How long is barn 1? _____ m or _____ ft

FS: Describe the air inlets on barn 1 in terms of size and distribution around the barn (e.g. 16 inch baffle board with a 12 inch opening along the entire length of one wall of the barn)?

_____

_____

FS: Describe the exhaust fans on barn 1 in terms of the number of fans of each diameter, and their location around the barn (e.g. five 36 inch diameter fans, ten 24 inch diameter fans, and three 14 inch diameter farms, located along one wall of the barn)?

_____

FS: Are there any circulating fans in barn 1?

_____ Yes (number, fan size and location in barn_____)

_____ No

FS: For flock _____, did you use misters, foggers or sprinklers in the barn?

_____ Yes – Misters/foggers

_____ Yes - Sprinklers

_____ No

FS: How many feed bins does barn 1 have? _____

BIOSECURITY

FS: What is the size of the CAZ around the study flock barn (e.g. 30 m, 100 ft)? _____

FS: For flock _____, was there an entrance room or anteroom in the barn? _____ Yes

_____ No

If no, describe the barn entrance area (e.g. direct access from outside) _____

FS: For flock _____, was there a distinct clean area and dirty area immediately outside the entrance to the RA? _____ Yes

_____ No

If yes, describe the barrier used to distinguish the clean side from the dirty side, and how often you used it?

_____ Painted line / marker

_____ < 25% of the time

_____ 25-50% of the time

_____ 51-75% of the time

_____ > 75% of the time

_____ Step-over (e.g. rope, wooden board)	_____ < 25% of the time _____ 25-50% of the time _____ 51-75% of the time _____ > 75% of the time
_____ Bench (e.g. wooden/plastic bench)	_____ < 25% of the time _____ 25-50% of the time _____ 51-75% of the time _____ > 75% of the time
_____ Straw bale	_____ < 25% of the time _____ 25-50% of the time _____ 51-75% of the time _____ > 75% of the time
_____ Door	_____ < 25% of the time _____ 25-50% of the time _____ 51-75% of the time _____ > 75% of the time
_____ Other (_____)	_____ < 25% of the time _____ 25-50% of the time _____ 51-75% of the time _____ > 75% of the time

If no, what measures did you use to prevent bringing infectious agents into the RA?

_____

_____

If the flock was from more than one barn, is the description of the entrance room and clean/dirty areas the same for the other barns?

_____ Yes

_____ No (*go to Part C*)

If the flock consisted of birds from more than 1 barn, were employees specific to each barn? _____ Yes
_____ No
_____ N/A – only 1 employee

GM: Do you manage or work on another poultry farm? _____ Yes
_____ No (*Check N/A in table for farm-specific clothing/footwear, page 9*)

If yes, do you access the RA on the other farm? _____ Yes
_____ No

FS: When flock _____ was in the barn, were there any visitors in the barn?
_____ Yes
_____ No (*Check No visitorsin table, page 7 AND cross out Visitors column*)

FS: Were there any service personnel in the barn other than chick delivery and catching crews (e.g. repair person, electrician, veterinarian, feed delivery guy)?
_____ Yes
_____ No (*Check No service personnelin table, page 7 AND cross out Service personnel column*)

FS: For flock _____, describe the biosecurity protocols that were used when entering the RA during grow-out for the following categories (*Check all that apply*):

Protocol	**Owner / Employees**	**Visitors**	**Service personnel**
Sign visitor's log book _____ No visitors or service personnel (Refer to above question regarding visitors)		_____ Always _____ Sometimes _____ No _____ Uncertain	_____ Always _____ Sometimes _____ No _____ Uncertain

Protocol	**Owner / Employees**	**Visitors**	**Service personnel**
Accompany visitors/service personnel when they accessed the RA _____ No visitors (Refer to above question regarding visitors) _____ No service personnel other than chick delivery or catching crew (refer to above question regarding service personnel or chick delivery)		_____ Always _____ Sometimes _____ No _____ N/A (visitor had knowledge of farm's biosecurity protocol) _____ Uncertain	_____ Always _____ Sometimes _____ No _____ N/A (visitor had knowledge of farm's biosecurity protocol) _____ Uncertain
<u>Footwear and/or boot dips</u> (e.g. dedicated boots or shoes disposable or plastic boots basin or tray with solution or powder, foam system, sprays, granular/crunch products) _____ N/A	_____ Always _____ Sometimes _____ No _____ Uncertain (for employees only)	_____ Always _____ Sometimes _____ No _____ Uncertain	_____ Always _____ Sometimes _____ No _____ Uncertain
<u>Clothing</u> (e.g. dedicated coveralls or clothing disposable suits clean clothing) _____ N/A	_____ Always _____ Sometimes _____ No _____ Uncertain (for employees only)	_____ Always _____ Sometimes _____ No _____ Uncertain	_____ Always _____ Sometimes _____ No _____ Uncertain

Protocol	**Owner / Employees**	**Visitors**	**Service personnel**
<u>Hats, caps, hair nets, or hoods on disposable suits</u> _____ Nothing worn on head	_____ Always _____ Sometimes _____ No _____ Uncertain (for employees only)	_____ Always _____ Sometimes _____ No _____ Uncertain	_____ Always _____ Sometimes _____ No _____ Uncertain
<u>Masks</u> _____ No masks used	_____ Always _____ Sometimes _____ No _____ Uncertain (for employees only)	_____ Always _____ Sometimes _____ No _____ Uncertain	_____ Always _____ Sometimes _____ No _____ Uncertain
<u>Hands</u> (e.g. hand washing, hand sanitizing, disposable gloves, barn specific gloves) _____ N/A	_____ Always _____ Sometimes _____ No _____ Uncertain (for employees only)	_____ Always _____ Sometimes _____ No _____ Uncertain	_____ Always _____ Sometimes _____ No _____ Uncertain
Other protocols _____ No other protocols	_____ Always _____ Sometimes _____ No _____ Uncertain	_____ Always _____ Sometimes _____ No _____ Uncertain	_____ Always _____ Sometimes _____ No _____ Uncertain
Farm (premise)-specific boots _____ N/A (only 1 farm)	_____ Always _____ Sometimes _____ No _____ Uncertain		

Protocol	Owner / Employees	Visitors	Service personnel
Farm (premise)-specific clothing _____ N/A (only 1 farm)	_____ Always _____ Sometimes _____ No _____ Uncertain		

If boot dips were used, how often was the dip changed?
_____ Daily
_____ Every other day, on average
_____ Twice per week
_____ Once per week
_____ Less frequent than once per week

If boot dips were used, describe dip, specify product(s) and describe how the products were used (e.g. rotate products every month)

_____

FS: Was flock _____ thinned? _____ Yes
_____ No

If yes, did the catchers wear premise-specific boots/clothing? _____ Yes
_____ No
_____ Uncertain

If yes, was the barn the first on the catching schedule? _____ Yes
_____ No
_____ Uncertain

FS: For flock _____, were there any dogs, cats or other pets (e.g. rabbits, pet birds) on the farm? _____ Yes
_____ No

If yes, <u>where were they allowed</u> on the farm? (*For each column, check only one row*)

Area where domestic animals were allowed	**Dogs**	**Cats**	**Other (_____)**
Entirely outside the CAZ			
Within the CAZ but never inside the barn			
Inside the entrance room or anteroom of the barn but not inside the RA			
Inside the RA			

FS: For flock _____, did you raise <u>any other livestock</u>? _____ Yes
_____ No

If yes, provide specific details of where the other livestock were kept? (*Check all that apply*)

Livestock	**At another location (specify distance)**	**On the same farm but in a <u>separate</u> barn**	**On the same farm and in the <u>same</u> barn**	***If in the same barn*, are they raised in the same RA?**
Cattle				
Sheep, goats				
Pigs				
Horses, donkeys				
Deer, llamas				
Rabbits				
Mink				
Other				

FS: For flock _____, did you raise <u>any other types of poultry</u>? _____ Yes
_____ No

If yes, provide specific details of where the other poultry were kept? (*Check all that apply*)

Poultry	**At another location (specify distance) (see below)**	**On the same farm but in a <u>separate</u> barn (see below)**	**On the same farm and in the <u>same</u> barn**	***If in the same barn*, are they raised in the same RA?**
Broiler breeders				

Poultry	**At another location (specify distance) (see below)**	**On the same farm but in a separate barn (see below)**	**On the same farm and in the same barn**	***If in the same barn*, are they raised in the same RA?**
Layers, including pullets and parent stock				
Turkeys, including parent stock				
Ducks, geese				
Pigeons				
Emu, ostrich, rheas				
Quail, pheasants				
Other				

If you raised other types of poultry at another location, did you have premise-specific employees?

_____ Yes
_____ No
_____ N/A – no other employees

If you raised other types of poultry on the same farm but in a separate barn, did you have barn-specific employees?

_____ Yes
_____ No
_____ N/A – no other employees

PEST CONTROL

FS: For flock _____, did you have a garbage bin (e.g. pail, bucket, can) in the barn? _____ Yes
_____ No

If yes, what items were in the garbage bin that might attract pests (e.g. food scraps, drink containers, floor sweepings, feed)?

_____

If yes, how often did you dispose of the garbage? _____ Twice daily
_____ Daily
_____ Every other day, on average
_____ Weekly
_____ Less frequent than once weekly

FS: For flock _____, did you have a feed spill outside the barn? _____ Yes (_____)
_____ No

If yes, was it cleaned up immediately? _____ Yes
_____ No (How long_____)

FS: For flock _____, were there any lights outside the barn? _____ Yes
_____ No

If yes, what type of lights did you have? _____ Flood lights
_____ Motion-activated lights
_____ Dawn-to-dusk-activated lights

FS: For flock _____, did you observe any wild animals other than wild birds (e.g. skunks, raccoons, possums, muskrats) in or near the barn? _____ Yes
_____ No

FS: For flock _____, were there any areas outside the barn that had stagnant water (including potholes)? _____ Yes
_____ No
_____ N/A – winter

GM: Do you have a bedding storage area on the farm? _____ Yes
_____ No

CLEANING AND DISINFECTION OF BARN, WORK ROOM / OFFICE, AND EQUIPMENT

FS: For flock _____, describe how you cleaned and disinfected the barn and equipment prior to chick placement?

_____

_____

_____

Keywords for C&D: hand sweep; scraping; removal of manure and organic matter; blow down; clean with water only; clean with detergent; disinfect with spray, foam or fog/fumigant; steam; contracted C&D company; squeegee

Keywords for surface types: feed pans; drinker lines; air inlets; exhaust fans; mortality buckets; barn-specific boots; catching equipment; small tractor/Bobcat

GM: Within the last year, how many times did you perform the following procedures? (*Check each column once*)

Number of times for each procedure	**Wash barn with high pressure**	**Wash equipment with high pressure**	**Disinfect barn (spray, foam, fog, steam)**	**Disinfect equipment (spray, foam, fog, steam)**
0				
1				
2				
3				
4				
5				
6				
≥ 7				

If you disinfected the barn or equipment more than once, did you change products? _____ Yes (_____)
_____ No

GM: Within the last year, did you clean/wash the exterior surfaces of the barn? _____ Yes (Number of times _____)
_____ No

If yes, describe the cleaning process, including what surfaces were cleaned:

_____

_____

WATER

FS: What was the water source for flock _____?

_____ Surface water (e.g. reservoir, pond, lake, river, rainwater collection)
_____ Well
_____ Municipal water supply
_____ Other (_____)

FS: Describe thedrinkers used for flock _____:

_____ Open - bell
_____ Open - trough
_____ Open -nipplewith cup catcher (e.g. Ziggity)
_____ Closed – nipple without cup catcher
_____ Other (_____)

If open drinkers were used, were any of the following problems noted? (*Check all that apply*)

_____ Slime
_____ Mold
_____ Scale
_____ Other (_____)

FS: For flock _____, did you place extra drinking water on the barn floor for the chicks at placement?

_____ Yes (_____)
_____ No

GM: In general, do you test the drinking water for bacterial contamination?

_____ Yes
_____ No

If yes, how often do you test?

_____ Once per year
_____ Twice per year
_____ More frequent than twice per year but not before every flock
_____ Before each flock

GM: Where in the system do you test the flock's drinking water? _____ At the source
_____ At the beginning of the water line in the barn
_____ At the end of the water line in the barn
_____ At the house
_____ Other (_____)

GM: Within the last year, how many times did you flush the water lines for routine cleaning? _____

GM: Describe your general procedure for flushing water lines for routine cleaning purposes, including water pressure, detergents, disinfectants, acids, descalers, or any other products

_____

_____

FS: For flock _____, did you flush the water lines or clean the nipples/drinkers during grow-out? _____ Yes
_____ No

If yes, describe how you flushed the lines and what products you used, including descalers_____

_____

FS: For flock _____, did you perform any on-farm water quality tests or visual inspections? _____ Yes
_____ No (_____)

If yes, describe the type oftests or inspectionsand frequency (*Check each column once*)

Frequency of testing/checking	**pH**	**Chlorine (total, free)**	**Turbidity**	**Cloudiness**	**Rust**	**Odour**	**Other (_____)**
Twice daily							
Once daily							
Every other day							
Once per week							
Once per two weeks							
Once per month							
Less frequent than once per month							
Not inspected or tested							

FS: For flock _____, were there any water quality problems (e.g. see table above)? _____ Yes
_____ No

If yes, what did you find and how did you solve the problem? _____

_____

FS: For flock _____, were there any water availability problems (e.g. pump failure, clogged nipples, dry well)? _____ Yes
_____ No

If yes, what was the cause and how did you solve the problem? _____

_____

BEDDING AND LITTER CONDITION

FS: For flock _____, what type of bedding was used? _____ Shavings
_____ Wood chips
_____ Chopped straw
_____ Long straw
_____ Other (_____)

FS: For flock _____, were the followinggeneral litter quality conditions observed at any time? (*Check all that apply*)

_____ No problem
_____ Too wet
_____ Dusty
_____ Moldy
_____ Uneven thickness
_____ Caked
_____ Matted
_____ Other (_____)

FS: For flock _____, were the following litter quality conditions observed under the water linesat any time? (*Check all that apply*)

_____ No problems
_____ Wet
_____ Moldy
_____ Uneven thickness
_____ Caked
_____ Matted
_____ Other (_____)

FEED AND FEED BINS

FS: For flock _____, what was the source of feed? (*Check all that apply*)

_____ Fresh feed – delivered from feed mill
_____ Fresh feed – picked up from feed mill
_____ Fresh feed - mixed on-farm or added ingredient on-farm
_____ Fresh feed - transfer from other farm(s)

_____ Leftover feed – from previous flock
_____ Leftover feed – transfer from another farm

If mixed on-farm, which of the following control programs were in place? (*Check all that apply*)

_____ Programs to monitor for bacterial contamination
_____ Programs to ensure proper nutritional balance

_____ No control program

If transferred from other farms, did the farms have a control program? _____ Yes
_____ No
_____ Uncertain

FS: For flock _____, at any time during grow-out, were there any of the following feed-related problems? (*Check all that apply*)
_____ No problems
_____ Mold
_____ Caking
_____ Insufficient feed in feeders
_____ Unsuitable form of feed (e.g. crumb, pellet) for age of flock
_____ Other (_____)

If more than one feed bin for any of the barns, did you use separate bins for medicated and non-medicated feed?
_____ Yes
_____ No

FS: Between flock _____ and flock _____, did you washthe feed bins/boot/auger? _____ Yes
_____ No

FS: Between flock _____ and flock _____, did you bang downthe feed bins? _____ Yes
_____ No

GM: Within the last year, how many times did you wash the feed bins/boot/auger? _____

GM: Within the last year, how many times did you bang down the feed bins? (*NB: related to eliminating medicated feed with a withdrawal period*) _____

_____

GM: Within the last year, did you encounter any of the following problems with your feed bins? _____ No problems
(*Check all that apply*) _____ Leaks

_____ Mold growth
_____ Other (_____)

BIRD ENVIRONMENT: TEMPERATURE, AIR QUALITY, AND LIGHTING

FS: For flock _____, did you place extra feed on the barn floor for the chicks at placement?
_____ Yes (_____)
_____ No

FS: For flock _____, how long was the brooding period? _____ days

FS: For flock _____, how old where the birds at shipping? _____ days

FS: For flock _____, describe the lighting schedule for each of the major periods outlined in the table: (*Check each column once*)

Maximum duration of light during each 24 hour period (full or at least 50% intensity)	**During brooding**	**During grow-out**	**Within 1 week of shipping**
24 hours			
23 hours (or 1 hour darkness)			
22 hours (or 2 hours darkness)			
21 hours (or 3 hours darkness)			
20 hours (or 4 hours darkness)			
19 hours (or 5 hours darkness)			
18 hours (or 6 hours darkness)			
17 hours (or 7 hours darkness)			
16 hours (or 8 hours darkness)			
<16 hours (or > 8 hours darkness)			

FS: For flock _____, did you observe any indicators of thermal discomfortduring brooding?(*Check all that apply*)
_____ Yes - huddling
_____ Yes - avoiding heat sources
_____ Yes - staying near heat sources

_____ Yes - other (_____)
_____ No

If yes, when (_____ age of birds in days) and for how long? _____ days

FS: For flock _____, did you observe any indicators of thermal discomfortduring the remainder of grow-out?(*Check all that apply*)
_____ Yes - huddling
_____ Yes - avoiding heat sources
_____ Yes - staying near heat sources
_____ Yes - other (_____)
_____ No

If yes, when (_____ age of birds in days) and for how long? _____ days

FS: For flock _____, which of the following monitoring and alarm systems were in place?(*Check all that apply*)
_____ Temperature outside of a pre-specified or set range
_____ Power failure
_____ Ventilation shutdown
_____ Water malfunction
_____ Feed malfunction (_____)
_____ High ammonia levels
_____ Other (_____)

FS: During grow-out, was the flock exposed to any of the following? (*Check all that apply*)
_____Temperature extremes
_____ Power failure without immediate generator back-up
_____ Ventilation shutdown
_____ Water deprivation
_____ Feed deprivation

If any of the above are checked, describe when (_____ age of birds in days) and for how long _____ days

If any of the above are checked, describe when (_____ age of birds in days) and for how long _____ days

If any of the above are checked, describe when (_____ age of birds in days) and for how long _____ days

MANURE MANAGEMENT

GM: Do you dispose of manure on farm, off farm, or both? _____

GM: Specify where you dispose of the manure (*Check all that apply*)

_____ On-farm - manure pile within CAZ
_____ On-farm - manure pile outside CAZ
_____ On-farm - compost barn within CAZ
_____ On-farm - compost barn outside CAZ
_____ On-farm - immediately spread on fields

_____ Off-farm – transported away from premises
_____ Other (specify _____)

If on-farm, what is the shortest distance between the study flock barn and the manure disposal area (e.g. 30 m, 100 ft)? _____

BIRD SUPERVISION, MORTALITY AND DISEASE MANAGEMENT

FS: For flock _____, on average, how many times per day did you monitor the flock and how long were you in the barn each time?

_____ 0
_____ 1 (Duration per visit _____minutes)
_____ 2 (Duration per visit_____ minutes)
_____ ≥ 3 (Duration per visit_____ minutes)

FS: For flock _____, on average, how many times per day did you collect mortalities? _____

FS: For flock _____, describe where and how you disposed of dead birds, including where the birds were placed immediately after collection, and their terminal location (*For each column, check all that apply*):

Location immediately after collection	**Terminal location**

_____ Inside RA	_____ On-farm - manure pile (*see below*)
_____ Inside entrance room or anteroom of barn	_____ On-farm - compost (*see below*)
_____ Inside freezer withinentrance room, workroom, or anteroom	_____ On-farm - incinerator
_____ Outside RA but inside CAZ	_____ Off-farm - transport to rendering plant
_____ Outside CAZ	_____ Off-farm – transport to another location
_____ Immediate incineration	
_____ Other (_____)	_____ Other (_____)

If the terminal disposal location was a <u>manure pile or compost on-farm</u>, how far was the terminal disposal location from the following items?

_____ Distance from feed sources (_____ m or _____ ft)
_____ Distance from watersource (_____ m or _____ ft) or _____ N/A

PART B: FORMS

PRODUCER INFORMATION

Registered Farm Name: _____

Farm Number: _____

Producer Name: _____

Producer Address: _____

Farm Location: Lot: _____ Township: _____

Concession: _____ County: _____

Farm Contact: _____ Manager: _____

FORM 6

Producer number (P #) _____

SHIPPING DETAILS

(*Complete one row for **each truckload**. If fewer than 6 truckloads, enter N/A for each row that is not applicable*):

Shipment number	**Form number**	**Date of shipment**	**Number of birds**
1			
2			
3			
4			
5			
6			
7			
8			
9			

Flock shipped at _____ days

***New forms*: CFC OFFSAP AND ACP FLOCK-SPECIFIC RECORDS (2009) FROM *PREVIOUS* QUOTA PERIOD**

***Old forms*: OFFSAP REQUIREMENTS PRIOR TO FLOCK PLACEMENT FOR *CURRENT* QUOTA PERIOD**

CLEANING AND DISINFECTION OF BARN, WORK ROOM/OFFICE, EQUIPMENT AND WATER LINES

FS: For flock _____, which of the following procedures did you carry out prior to chick placement?

Water temperature used during barn cleaning	_____ Cold _____ Hot
Water pressure used during barn cleaning	_____ Low _____ High (i.e. power washer)
Barn Cleaning products (soaps, detergents, sanitizers, NOT disinfectants)	_____ Yes (product/date_____) _____ No
Barn Presoaking	_____ Yes (_____) _____ No
Barn Fumigation	_____ Yes (product/date_____) _____ No
Barn Disinfection (other than fumigation)	_____ Yes (product/date/method of administration _____) _____ No

FS: Between flock _____ and flock _____, did you flush the water lines? _____ Yes (product/date _____)
_____ No

If yes, did you flush the water lines more than once? _____ Yes
_____ No

If yes, describe the flushing procedure, including how many times, how many days prior to chick placement, and the products used for each flushing?

_____

FS: Between flock _____ and flock _____, how long was the rest period (i.e. period between last day of cleaning/disinfection and chick placement)? (*New forms only*) _____ days

PEST CONTROL

For flock _____, did you use rodent traps? _____ Yes
_____ No

If yes, where were the traps located? _____

For flock _____, did you use rodenticides (bait stations)? _____ Yes
_____ No

If yes, where were the bait stations located? _____

Fly traps (Fly control) _____ Yes – new
_____ Yes – from previous flock
_____ No

Fly screens (Fly control) _____ Yes
_____ No

Insect foggers/sprays _____ Yes
(products/dates _____) _____ No

Insecticide powder along wall-floor junction _____ Yes
(products/dates _____) _____ No

Insect zapper (UV light) _____ Yes
_____ No

Fly fungus	_____ Yes _____ No
Holes in barn walls, roof and doors repaired	_____ Yes _____ No _____ N/A – holes were not present
Cracks in floors repaired	_____ Yes _____ No _____ N/A – cracks were not present
Grass cut around barn	_____ Yes _____ No _____ N/A – winter _____ N/A – concrete or gravel or crushed rock

***New forms*: CFC OFFSAP AND ACP FLOCK-SPECIFIC RECORDS (2009) FROM *CURRENT* QUOTA PERIOD**

***Old forms*: OFFSAP REQUIREMENTS DURING GROW-OUT FOR *CURRENT* QUOTA PERIOD**

WATER

FS: For flock _____, did you treat the drinking water during grow-out? _____ Yes
_____ No
_____ N/A - water is treated by municipality

If yes, what type of treatmentwas used and howoften:

Type of water treatment	**Continuous treatment**	**Intermittent treatment (specify how often)**
Reverse osmosis		
Ultraviolet light		
Chlorine (_____ ppm)		
Hydrogen peroxide (_____)		
Ozone		
Acid (_____)		
Ultra-filtration		
Other (_____)		

FS: *If using open drinkers,* how often were the drinkers disinfected during the life of the flock?
_____ Routinely (dates_____)
_____ As needed (dates_____)
_____ Not disinfected

FS: During grow-out, did you check for water availability (e.g. dry well, adequate pressure, patency of nipples)? _____ Yes
_____ No

If yes, how often? _____ Continuously
_____ Twice daily

_____ Once daily
_____ Every other day
_____ Once per week
_____Less frequent than once per week

FS: During grow-out, did you carry out daily feed checks? _____ Always
_____ Sometimes
_____ No

BIRD ENVIRONMENT: TEMPERATURE, AIR QUALITY, AND LIGHTING

FS: Describe how you monitored barn temperature (*complete table below*)

Location of monitoring	**Frequency of monitoring**
_____ Bird level _____ Other	_____ Continuous _____ Twice daily _____ Once daily _____ At least once per week _____ At least once per two weeks _____ At least once per month _____ At least once during grow-out

FS: During grow-out, did you monitor humidity levelswith a device or by monitoring compaction of the litter and dust level?
_____ Yes (*complete table below*)
_____ No

FS: Describe how you monitored humidity: (*Complete all rows that apply*)

Method of monitoring	**Frequency of checking humidity levels**	**Humidity outside of normal range (50-70%), or notable compaction of the litter or high dust, at any time during grow-out**

Method of monitoring	**Frequency of checking humidity levels**	**Humidity outside of normal range (50-70%), or notable compaction of the litter or high dust, at any time during grow-out**
Hand-held device	_____ Twice daily _____ Daily _____ At least once per week _____ At least once per two weeks _____ At least once per month _____ At least once during grow-out	_____ Yes – too high (age - _____ days; duration - _____ days) _____ Yes – too low (age - _____ days; duration - _____ days) _____ No
Permanently-installed humidity meter	_____ Twice daily _____ Daily _____ At least once per week _____ At least once per two weeks _____ At least once per month _____ At least once during grow-out	_____ Yes – too high (age - _____ days; duration - _____ days) _____ Yes – too low (age - _____ days; duration - _____ days) _____ No
Compaction of the litter and level of dust	_____ Twice daily _____ Daily _____ At least once per week _____ At least once per two weeks _____ At least once per month _____ At least once during grow-out	_____ Yes – too high (age - _____ days; duration - _____ days) _____ Yes – too low (age - _____ days; duration - _____ days) _____ No

FS: During grow-out, did you monitor ammonia levels with a device or by smell or eye irritation? _____ Yes (*complete table*)
_____ No

FS: Describe how you monitored ammonia levels: (*Completeall rows that apply)*

Method of monitoring	**Location of monitoring**	**Frequency of monitoring**	**Ammonia level > 25 ppm, or notable nose/eye irritation, at any time during grow-out**

Method of monitoring	**Location of monitoring**	**Frequency of monitoring**	**Ammonia level > 25 ppm, or notable nose/eye irritation, at any time during grow-out**
Hand-held device	_____ Bird level _____ Other	_____ Twice daily _____ Daily _____ At least once per week _____ At least once per two weeks _____ At least once per month _____ At least once during grow-out	_____ Yes (age - _____ days; duration - _____ days) _____ No
Permanently-installed ammonia meter	_____ Bird level _____ Other	_____ Twice daily _____ Daily _____ At least once per week _____ At least once per two weeks _____ At least once per month _____ At least once during grow-out	_____ Yes (age - _____ days; duration - _____ days) _____ No
Smell/eye irritation	_____ Bird level _____ Other	_____ Twice daily _____ Daily _____ At least once per week _____ At least once per two weeks _____ At least once per month _____ At least once during grow-out	_____ Yes (age - _____ days; duration - _____ days) _____ No

BARN ENVIRONMENT

FS: Entrance rooms and work rooms cleaned and free of debris/dust

_____ Twice daily
_____ Daily
_____ Every other day, on average
_____ Weekly
_____ Less frequent than once weekly

FORM 3

HATCHERY RECORD

FEED INVOICES

FLOCK CHARACTERISTICS

FS: Breed and strain of flock _____

FS: Gender: _____ Pullets _____ Cockerels _____ Mixed

FS: Source of chicks ((Name of hatchery): _____

FS: Date of chick placement: _____ FS: Number of chicks placed: _____

FS: Age of Breeder Flock: _____

FS: Target market weight: (Average weight _____ at _____ days)

VACCINATION HISTORY

Vaccinations given <u>at the hatchery</u>

Name of vaccine and dosage	Method of administration	Date of administration
1	_____ In-ovo _____ Injection _____ Spray	
2	_____ In-ovo _____ Injection _____ Spray	

Name of vaccine and dosage	Method of administration	Date of administration
3	_____ In-ovo _____ Injection _____ Spray	
4	_____ In-ovo _____ Injection _____ Spray	

Vaccinations given <u>during grow-out</u>

Name of vaccine and dosage	Reason for administration	Method of administration	Date of administration	Person who administered
1	_____ Prevention _____ Outbreak	_____ Puck _____ Water _____ Spray – coarse _____ Spray – fine _____ Ocular _____ Wing web _____ Other(_____)		_____ Producer _____ Employee _____ Veterinarian _____ Drug representative _____ Other(_____)
2	_____ Prevention _____ Outbreak	_____ Puck _____ Water _____ Spray – coarse _____ Spray – fine _____ Ocular _____ Wing web _____ Other(_____)		_____ Producer _____ Employee _____ Veterinarian _____ Drug representative _____ Other(_____)
3	_____ Prevention _____ Outbreak	_____ Puck _____ Water _____ Spray – coarse _____ Spray – fine _____ Ocular _____ Wing web _____ Other(_____)		_____ Producer _____ Employee _____ Veterinarian _____ Drug representative _____ Other(_____)

Name of vaccine and dosage	**Reason for administration**	**Method of administration**	**Date of administration**	**Person who administered**
4	_____ Prevention _____ Outbreak	_____ Puck _____ Water _____ Spray – coarse _____ Spray – fine _____ Ocular _____ Wing web _____ Other(_____)		_____ Producer _____ Employee _____ Veterinarian _____ Drug representative _____ Other(_____)

BIRD SUPERVISION, MORTALITY, DISEASE MANAGEMENT AND MEDICATION HISTORY

Disease and treatments during the grow-out period (*Form 3*)

Name of disease or syndrome	**Name of medications (trade or brand name)**	**Dosage**	**Method of administration (water/feed)**	**Safe marketing date as per recommended withdrawal period**	**Date of first treatment**	**Date of last treatment**	**Flock recovered (Y/N)**	**Veterinary diagnosis or confirmed (Y/N)?**	**Percent mortality due to disease (*ask)*
	1								
	2								
	3								
	4								

Water Medication Management(*Medication number must match Table above*)

Medication #	**Person who administered**	**Medicator checked for proper functioning before use**	**Water lines flushed <u>before</u> treatment**	**Water lines flushed <u>after</u> treatment**
1	_____ Producer _____ Employee _____ Vet _____ Drug rep _____ Other (_____)	_____ Yes (*see below*) _____ No	_____ Yes _____ No	_____ Yes _____ No
2	_____ Producer _____ Employee _____ Vet _____ Drug rep _____ Other (_____)	_____ Yes _____ No	_____ Yes _____ No	_____ Yes _____ No

Medication #	**Person who administered**	**Medicator checked for proper functioning before use**	**Water lines flushed <u>before</u> treatment**	**Water lines flushed <u>after</u> treatment**
3	_____ Producer _____ Employee _____ Vet _____ Drug rep _____ Other (_____)	_____ Yes _____ No	_____ Yes _____ No	_____ Yes _____ No
4	_____ Producer _____ Employee _____ Vet _____ Drug rep _____ Other (_____)	_____ Yes _____ No	_____ Yes _____ No	_____ Yes _____ No

If a medicator was used, how did you calibrate it? _____

_____

Name of feed mill _____

Feed Medication Table

Feed	**Names of medication (trade or brand name)**	**Dosages**	**Withdrawal periods (days)**
1 _____ Start date _____ End date			
2 _____ Start date _____ End date			
3 _____ Start date _____ End date			

Feed	Names of medication (trade or brand name)	Dosages	Withdrawal periods (days)
4 _____ Start date _____ End date			
5 _____ Start date _____ End date			
6 _____ Start date _____ End date			
7 _____ Start date _____ End date			

Feed	Names of medication (trade or brand name)	Dosages	Withdrawal periods (days)
8 _____ Start date _____ End date			
9 _____ Start date _____ End date			
10 _____ Start date _____ End date			
11 _____ Start date _____ End date			
12 _____ Start date _____ End date			

FS: For flock _____, describe the methods used to prevent cross-contamination from medicated feedwith a withdrawal period to the next type of feed (*Check all that apply*)

Single bins	Double bins

Single bins	**Double bins**
_____ Feeding lines run empty before next feed _____ Rubber mallet to knock the sides of the feed bin before each new load of feed _____ Other (_____) _____ No method used	_____ Feeding lines run empty before next feed _____ Rubber mallet to knock the sides of the feed bin before each new load of feed _____ Complete emptying of feed bin and auger before switching to the next type of feed _____ Other (_____) _____ No method used

For the methods described above, how often did you use the methods?

_____ After every feed regardless of whether it was medicated or not
_____Only after feeds containing medication
_____ Only after medicated feeds containing drugs with a withdrawal period
_____ Other (_____)

<u>Bacteriological Analysis of drinking water for Private Citizen</u>

Total Coliform Count per 100 ml _____ Date tested _____

E. coli per 100 ml _____