Association of increased rate of condemnation of broiler carcasses due to hepatic abnormalities with immunosuppressive diseases in the broiler chicken industry in Saskatchewan

Keyvan Amini, Tara Zachar, Shelly Popowich, Tennille Knezacek, Bob Goodhope, Philip Willson, Susantha Gomis

Introduction

Macroscopic pathological changes in broiler carcasses lead to condemnation at the time of processing. Such changes include lesions of the skin, subcutaneous tissue, liver, air sacs, and joints, as well as general disease manifestations such as ascites, emaciation, and cyanosis (1). According to data published by the United States Department of Agriculture, in 2010 approximately 150 200 metric tons of chicken meat, or 0.88% of the total chicken meat production in the United States, was rejected as a result of carcass condemnation (2). Data from Agriculture and Agri-Food Canada (AAFC) indicate that in 2010 an average of 1.08% of broilers were condemned at the time of processing because of various pathological changes, and between 1999 and 2009 the main cause of condemnation (accounting for increased rate of condemnation of broiler carcasses due to hepatic abnormalities with immunosuppressive diseases in the broiler chicken industry in Saskatchewan

Abstract

The objective of this study was to identify the causative agents of hepatitis observed in broiler chickens at processing. Livers of chickens from 16 broiler farms in Saskatchewan with gross lesions of hepatitis were collected at processing. In addition to routine bacterial isolation and histopathological examination, serologic studies for infectious bursal disease virus (IBDV) and Chicken anaemia virus (CAV), calculation of the ratio of the weight of the bursa of Fabricius (BF) to body weight (BBW), and histopathological examination of the BF were done. Of the 264 livers with gross lesions, 83% had multifocal to coalescing necrotizing hepatitis, 16% had perihepatitis, and 1% had hemorrhages. No definitive causative microorganisms were isolated from the hepatic lesions; however, no significant bacterial isolations were made. Bursal atrophy, low BBW ratio, and high titer of antibody against IBDV each correlated with the rate of total condemnations ($P = 0.0188$, $P = 0.0001$, and $P = 0.0073$, respectively). Nucleotide sequencing of IBDV isolated from the BF identified the variant strains Delaware-E and 586. Condemnation because of hepatic lesions was correlated with titer of antibody against IBDV and BBW ($P = 0.016$ and $P = 0.027$). The results of this study demonstrate that hepatic lesions in Saskatchewan chickens are not currently caused by a primary bacterial pathogen but are associated with indicators of immunosuppression that is likely due to variant IBDV.

Résumé

L’objectif de la présente étude était d’identifier les agents causaux de l’hépatite observée chez des poulets à griller au moment de la transformation. Les foies de poulets provenant de 16 fermes de poulets à griller en Saskatchewan avec des lésions macroscopiques d’hépatite furent prélevés. En plus de l’isolement bactérien de routine et de l’examen histopathologique, on effectua des analyses sérologiques pour le virus de la bursite infectieuse aviaire (VBIA) et le virus de l’anémie du poulet (VAP), le calcul du ratio du poids de la bourse de Fabricius (BF) sur le poids corporel (BPC), et l’examen histopathologique de la BF. Sur les 264 foies ayant des lésions macroscopiques, 83 % avaient des lésions multifocales à coalescentes d’hépatite nécrosante, 16 % de la péri-hépatite et 1 % des hémorragies. Aucun agent causal définitif ne fut isolé des lésions hépatiques; toutefois, aucun agent bactérien significatif ne fut isolé. Une atrophie de la bourse, un faible ratio BPC, et un titre élevé d’anticorps dirigé contre VBIA correspondaient aux lésions de condamnation totale ($P = 0.0188$, $P = 0.0001$, et $P = 0.0073$, respectivement). Le séquençage nucléotidique des VBIA isolés des BF identifia les souches variantes Delaware-E et 586. La condamnation due aux lésions hépatiques était corrélée avec le titre d’anticorps contre VBIA et le BPC ($P = 0.016$ et $P = 0.027$, respectivement). Les résultats de la présente étude démontrent que les lésions hépatiques chez les poulets de la Saskatchewan ne sont pas actuellement causées par un agent bactérien pathogène primaire mais sont associées à des indicateurs d’immunosuppression qui est probablement causée par un variant de VBIA.

(Traduit par Docteur Serge Messier)
for 0.73% of condemnations) in federally inspected processing plants in Canada was subcutaneous conditions, also referred to as cellulitis (3). One manifestation of cellulitis in broiler chickens is fibrinocaseous exudates in the subcutaneous tissues of the percloacal area, from which *Escherichia coli* is the species most frequently isolated (4). Between 2006 and 2011, condemnations due to hepatic conditions ranked second across Canada (3). In 2010, hepatic conditions resulted in 0.10% of carcass condemnations at the national level and 0.22% of condemnations at the regional (Saskatchewan and Manitoba) level (3). Increased rates of carcass condemnation as a result of liver abnormalities have been reported in Norway (5) and England (6). Although the rate of condemnation due to hepatic conditions increased in the Canadian Prairies over the last decade (3), no specific etiologic agent was identified.

Infectious bursal disease virus (IBDV), which belongs to the genus *Avibirnavirus* of the family *Birnaviridae*, causes IBD, a major immunosuppressive disease among young chickens worldwide. Immunosuppressive agents such as IBDV can decrease flock performance, contribute to secondary infection, and increase the incidence of carcass condemnation (7,8). The most severe consequence of an IBDV infection is functional impairment of the bursa of Fabricius (BF). Uniform vaccine-induced titers of antibody against IBDV in broiler breeders have been associated with good performance by their progeny (9–11). The virus is very resistant to most disinfectants and environmental factors and persists for months in contaminated barns, water, feed, and droppings (12,13). One of the most important avian-acquired immunosuppressive diseases (8), IBD has led to large economic losses to the poultry industry worldwide.

A synergistic effect between IBDV and *Chicken anaemia virus* (CAV) has been reported (14). Infections with CAV occur worldwide and are commonly found in the commercial broiler chicken industry (15). Clinical disease results in increased rates of weight loss and death among broilers (16). Subclinical infections account for higher rates of feed conversion and reduced daily weight gain (17). Infections with CAV in broilers are also associated with increased condemnation rates (18,19).

The objectives of this study were to investigate whether hepatic lesions observed in broiler chickens at processing are caused by a primary etiologic agent and to identify the etiologic agent(s), including common immunosuppressive agents that are associated with hepatic lesions in these chickens.

**Materials and methods**

**Sample collection, histopathological study, and bacterial and viral isolation**

Sixteen broiler farms or premises (in 16 locations) among the 57 broiler farms in Saskatchewan were convenience-selected to represent the population of broiler farms in the province. All broilers in Saskatchewan were supplied by 2 hatcheries and were processed by 2 processing plants. The broilers at 19% of the included farms were vaccinated against IBDV with Clonevac D-78 (Intervet Canada, Whitby, Ontario), at 19% against both IBDV and Marek’s disease virus (MDV) (with Maraxine + SB1, Intervet Canada), and at 25% against *Avian infectious bronchitis virus* (IBV) (with MildVac-M, Intervet Canada); the broilers at the remaining 37% of the farms were not vaccinated against IBDV, MDV, or IBV. Data collected for each of the 16 farms at processing included bird age, average body weight, number of condemnations, and reason for condemnation, such as cellulitis, hepatitis, ascites, and airsacculitis.

Over the 12-month period of April 2009 to April 2010, 264 livers of chickens from the 16 broiler farms with gross lesions associated with carcass condemnation were randomly collected and described. The carcasses had been condemned at the 2 processing plants by the Canadian Food Inspection Agency (CFIA) according to the criteria outlined in chapter 19 of the Poultry Inspection Programs (1). In addition, 20 grossly normal livers from processed carcasses were randomly collected from both processing plants as control samples. The livers were shipped on ice to the Prairie Diagnostic Services, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, where 35 of those with macroscopic lesions and 8 of the grossly normal livers were randomly selected for bacterial culture under aerobic and anaerobic conditions. The colonies were quantified as none, few, or 1+ to 4+ by standard bacteriologic and biochemical methods (20,21). Another 26 of the livers with macroscopic lesions were processed for detection of *Campylobacter* species by polymerase chain reaction (PCR) and culture; no normal livers were included in this analysis. Tissue sections from all 264 livers with gross lesions and all 20 normal livers were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. In addition, 20 of the paraffin-embedded tissue sections from diseased livers were selected at random for Gram’s, periodic-acid–Schiff, and acid-fast staining. Virus isolation was attempted for 17 of the 264 livers with macroscopic lesions at the Animal Health Laboratory (AHL), University of Guelph, Guelph, Ontario.

**Serologic and BF studies**

Blood samples were collected at the time of processing from 20 birds in each of 2 broiler flocks from 13 of the 16 selected broiler farms. Titers of antibody against IBDV and CAV were measured at the AHL by commercial enzyme-linked immunosorbent assay (IDEXX, Westbrook, Maine, USA) according to the manufacturer’s instructions. After blood collection and euthanasia, the ratio of BF weight to body weight (BBW) was measured for each of the 520 birds. The BBW ratio is a good indicator of broiler performance and bursal atrophy due to IBDV (22). The BFVs were collected and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for histopathological examination. A score representing the degree of bursal atrophy was assigned as follows: 0 — normal; 1+ — mild atrophy; 2+ — moderate atrophy; and 3+ — severe atrophy (Figures 1A to 1D). A cumulative score was determined for each farm. The BFVs of chickens processed at day 19 of age from 4 randomly chosen farms were submitted to the AHL for virus isolation and nucleotide sequencing of IBDV.

**Statistical analysis**

For descriptive statistics and statistical analyses Prism software (version 5.03; GraphPad Software, San Diego, California, USA) was used. The geometric mean of non-normally distributed data
(antibody titers) was calculated to indicate the central tendency. Correlations of carcass condemnation with BBW ratio, bursal atrophy score, and IBDV and CAV titers were analyzed by Spearman’s rank test (nonparametric correlation). Significance was set at $P < 0.05$.

**Results**

The average chicken age at processing for the 16 farms included in the study was 37 d (range: 34 to 42 d) and the average live body weight 2.04 kg (range: 1.78 to 2.36 kg). According to the data collected from both processing plants, the average body weight was 2.17 kg (range: 1.49 to 2.67 kg) for the Saskatchewan broiler chicken industry during the study period. Among the 16 studied farms, the total rate of condemnation over the study period averaged 1.40% (range: 0.35% to 4.31%); the rate of condemnation due to subcutaneous and hepatic conditions averaged 0.83% (0.10% to 4.01%) and 0.37% (0.001% to 1.60%), respectively. Every flock studied had some birds whose carcass was condemned because of subcutaneous or hepatic lesions.

Of the 264 condemned livers studied, 218 (83%) had hepatitis that ranged from mild, acute through moderate, subacute to severe, chronic and was characterized by enlargement of the liver with multifocal through locally extensive to diffuse yellowish to white necrotic areas 2 to 4 mm in diameter (Figures 2A to 2C); 43 (16%) had perihepatitis (Figure 2D), and 3 (1%) had extensive areas of hemorrhage suggestive of trauma. The macroscopic lesions were widespread in both subcapsular areas and parenchyma. Histopathological examination of the 218 livers with hepatitis showed necrotic centers (Figure 2E) with proteinaceous material and cell debris surrounded by heterophils, lymphocytes, macrophages, and giants cells (Figure 2F), along with focal areas of amorphous proteinaceous material with very few fibroblasts and areas of expansion of extramedullary granulopoiesis (Figure 2G).
Histopathological examination of the 43 livers with perihepatitis revealed moderate to severe thickening of the hepatic capsule with fibrin and fibrosis, as well as infiltration with variable numbers of inflammatory cells, including macrophages, lymphocytes, and giant cells, along with fibroblasts (Figure 2D). The livers with perihepatitis had multifocal areas of expansion of extramedullary granulopoiesis (Figure 2H). Microscopically, the 3 livers with gross hemorrhage had locally extensive severe hemorrhage. No viral inclusion bodies, bacteria, acid-fast microorganisms, or fungal or parasitic agents were observed in the stained sections. No microscopic lesions were seen in any sections of the grossly normal livers.

Of the 35 livers with gross lesions that were cultured for bacteria, 27 (77%) yielded few to 1+ bacteria (Table I). No Campylobacter or Salmonella species were isolated. No bacteria were isolated from the 8 grossly normal livers that were cultured. No Campylobacter species were identified by PCR in the 26 livers with gross lesions that were tested. No virus was isolated from any of the 17 livers with gross lesions that were tested.

At the 13 farms studied for immunosuppressive agents, BBW ratio, and histopathological features of the BF, the geometric mean titer of antibody against IBDV was 1601 (range: 2 to 7467), the geometric mean titer of antibody against CAV was 1134 (range: 999

Figure 2. Macroscopic and microscopic appearance of livers condemned at processing. A — mild acute hepatitis, with multifocal yellowish to white necrotic areas 2 to 4 mm in diameter. B — subacute moderate hepatitis, with multifocal to locally extensive spots of the same size. C — severe chronic hepatitis, with locally extensive to diffuse spots of the same size. D — locally extensive moderate to severe chronic perihepatitis. E — multifocal necrotic centers of C; original magnification × 4. F — higher magnification of E; necrotic focus containing cell debris surrounded by macrophages and giant cells, with expansion of extramedullary granulopoiesis (arrow); original magnification × 10. G — higher magnification of an area with expansion of extramedullary granulopoiesis in a liver with hepatitis; original magnification × 40. H — higher magnification of an area with expansion of extramedullary granulopoiesis in a liver with perihepatitis; original magnification × 40. H&E staining.
Acinetobacter spp. Few to 1
Escherichia coli
Lactobacillus spp.
Staphylococcus spp.
Pasturella spp.
Enterococcus spp.
Bacteroides spp.
Enterobacter spp.
Acinetobacter spp.
Arcobacter spp.
Clostridium spp.

Table I. Results of anaerobic and aerobic culture of bacteria in 27 condemned livers among 35 with gross lesions selected at random

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Bacterial load</th>
<th>Number of livers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>Few to 1+</td>
<td>21</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>Few to 1+</td>
<td>9</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>1+</td>
<td>5</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>Few</td>
<td>3</td>
</tr>
<tr>
<td>Pasteurella spp.</td>
<td>Few to 1+</td>
<td>3</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>Few to 1+</td>
<td>3</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>1+</td>
<td>3</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>Few</td>
<td>2</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>1+</td>
<td>2</td>
</tr>
<tr>
<td>Arcobacter spp.</td>
<td>1+</td>
<td>1</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>1+</td>
<td>1</td>
</tr>
</tbody>
</table>

to 1641), and the average BBW ratio was 0.12 (range: 0.04 to 0.19). Histopathological examination of the BF yielded scores that varied from 0 (normal) to 3+ (severe atrophy) (Figures 1A to 1D). The cumulative score per farm averaged 19 (range: 5.0 to 58). Variant IBDV was identified at each of the 4 farms randomly selected for virus isolation from the BF: the 586 variant at 2 farms, the Delaware-E variant at 1 farm, and both variants at 1 farm.

The rate of total condemnations correlated with the rate of condemnation due to hepatic lesions ($P = 0.0085$; $r = 0.644$), bursal atrophy ($P = 0.0188$; $r = 0.649$), BBW ratio ($P = 0.0001$; $r = -0.890$), and titer of antibody against IBDV ($P = 0.0073$; $r = 0.720$). The rate of hepatic condemnation correlated with the titer of antibody against IBDV ($P = 0.016$; $r = 0.665$) and the BBW ratio ($P = 0.027$; $r = -0.621$). The titer of antibody against IBDV did not correlate with IBDV vaccination at the broiler farms ($P = 0.19$; $r = 0.30$).

Of the 13 farms, 4 (31%) had low BBW ratios (average 0.05); the remaining 9 had high (normal) BBW ratios (average 0.16). The farms with low BBW ratios had greater cumulative bursal atrophy scores, titers of antibody against IBDV, and rates of total condemnations due to subcutaneous and hepatic conditions than did the farms with high BBW ratios (Table II). The titer of antibody against CAV correlated with the titer of antibody against IBDV ($P = 0.016$; $r = 0.663$) and the rates of total condemnations ($P = 0.055$; $r = 0.549$) and hepatic condemnations ($P = 0.015$; $r = 0.669$).

Discussion

The proportion of broiler carcasses condemned because of hepatic lesions increased over the last decade at both regional and national levels. In Canada in 2010 more than 622 million broiler chickens were processed, and 1.08% were condemned at the time of processing owing to various lesions (3). The proportion of broilers condemned in Canada because of hepatic lesions remained higher than 0.10% from 2006 to 2011, fell below 0.06% in 2012, and then rose in 2013 to levels seen during 2006 to 2011 (3). The average body weight of the broilers from the farms selected for this study (2.0 kg) was comparable to that of the overall Saskatchewan broiler chicken industry during the study period (2.17 kg). In this study we showed an association between condemnation of carcasses as a result of hepatic conditions and indicators of immunosuppression.

Various types of hepatic lesions in chickens have been described. Hutchison and Riddell (23) described 2 types of lesions: 1 type, which they named “hepatitis,” was characterized by an enlarged, pale, and firm liver, often associated with a thickened gallbladder wall and distended bile ducts; the other type, described as “bosselated,” consisted of a swollen liver with grey discoloration and ascites. Randell et al (6) described enlarged, pale livers mottled with small yellow stellate foci beneath the capsule and throughout the parenchyma, associated with a thickened and distended gallbladder. They described the microscopic changes as those of chronic fibrosing hepatitis with bile duct proliferation and infiltration of immature granulocytes and reported the isolation of a few bacterial species, including E. coli, Clostridium perfringens, and Pasteurella haemolytica. According to Ito et al (24) a yellowish or whitish color of the liver is related to hepatocyte vacuolar degeneration and is likely associated with toxigenic injury, whereas focal or diffuse pinpoint or nodular lesions in the liver are caused by bacterial agents. We did not see any of the above-described lesions in our study. Macroscopically the condemned livers were friable, and intact areas were normal in color. Microscopically the livers had focal, multifocal, or multifocally coalescing areas of necrotic centers with cellular debris and infiltration of heterophils and lymphocytes. Some necrotic centers were surrounded by macrophages and giant cells. Although necrotic foci were numerous in livers with moderate to severe hepatitis, the remaining hepatic parenchyma was normal except for areas of expansion of extramedullary granulopoiesis and expansion of ectopic lymphoid aggregates. The hepatocytes were normal in unaffected areas, showing no vacuolation or atrophy. There was no bile duct proliferation or fibrosis around the bile ducts. Although necrotic foci were numerous in livers with subacute to chronic hepatitis, areas of fibrosis were minimal. It appears that these livers were battling with constant bacterial infections, but their regenerative capacity was high because damage to the hepatic architecture was minimal. The liver lesions in this study ranged from moderate to severe, locally extensive to diffuse, chronic active hepatitis with inconsistent low numbers of bacterial isolations. Hence liver lesions suggest an underlying primary disease.

More than 200 species of Gram-positive, Gram-negative, aerobic, and anaerobic bacteria are normally found in the intestines of chickens (25,26). Thus, the species of bacteria isolated in this study are considered normal intestinal flora in chickens (26–30). It is well-established that intestinal capillaries can transport bacteria and toxins to the liver through the portal system, and bacteria and endotoxins are a normal constituent of portal venous blood (31,32). Normally, intestinal toxins and bacteria that enter the liver through the portal system are eliminated by the Kupffer cells and reticuloendothelial cells of the liver (32–34). However, in immunosuppressed birds, as with humans and other animals, the clearance system is impaired, and the bacteria and endotoxins in the portal blood may induce hepatocyte injury. Continual antigenic stimulation of the liver as a result of an imbalance in the gut microflora or abnormal responses to the antigens because of impairment of the immune system can result in a prolonged inflammatory response and hepatitis, manifested as granulomas in the liver (34). Infectious bursal disease...
may precipitate a variety of conditions that might play a role in airsacculitis, septicemia, toxemia, increased rate of condemnation, and poor broiler performance (35). We hypothesize that livers from the immunosuppressed birds in our study were not able to clear bacteria and toxins from their intestines efficiently, the result being subacute to chronic hepatitis. We need to prove this hypothesis by experimental infection of broiler chickens with variant IBDVs isolated from the Canadian broiler chicken industry.

By causing immunosuppression in young chickens, IBDV makes these birds susceptible to secondary infections. In this study we found a high titer of antibody against IBDV, bursal atrophy, and a low BBW ratio at processing and isolated variant IBDVs from the BF. It has been reported that a low BBW ratio indicates immunosuppression (11,22). Indeed, we found that the titer of antibody against IBDV correlated with the BBW ratio and bursal atrophy. The farms with high titers of antibody against IBDV, low BBW ratios, and high bursal atrophy scores also had high total rates of condemnation, including condemnation due to hepatic lesions. No correlation was noted between the titer of antibody against IBDV and broiler vaccination.

Ojkic et al (36) reported that variant IBDVs are circulating in Canadian broiler chicken flocks and are responsible for immunosuppression. It has also been demonstrated that variant IBDVs suppress the humoral response and result in vaccination failure (8). The cellular immune system, including antigen-specific T-cells, were found to be severely compromised during IBDV infection (7). Moreover, Craft et al (37) demonstrated that variant IBDVs were more detrimental to the cell-mediated immune system than classic strains of IBDV. Further experimental studies are necessary to prove our hypothesis that susceptibility of broiler chickens to hepatitis increases after variant IBDV infection in the broiler chicken industry in Saskatchewan. We hypothesize that CAV infection was secondary to primary immunosuppression due to IBDV, thus explaining the relationship between the titers of antibody against CAV and IBDV observed in our study. However, the effect of CAV on hepatic condemnations needs to be examined by further epidemiologic and experimental studies.

According to current AAFC guidelines, the entire chicken carcass is condemned in cases of hepatitis, especially those with moderate to severe hepatic lesions, because of the possibility of septicemia and associated bacterial contamination of the carcass. In this study the birds with hepatitis were not septicemic; in fact, very low numbers of bacteria were isolated. Thus, we suggest that only the inflamed liver be condemned and not the entire carcass.

In conclusion, this study has demonstrated that hepatic lesions are not caused by a primary bacterial pathogen but are likely associated with elevated titers of antibody against IBDV and concurrent immunosuppression. It is important to evaluate current programs of vaccination against IBDV in the broiler breeder industry to control current variant IBDV infections in broilers.

Acknowledgments

The authors are grateful to staff at the Saskatoon and Wynyard poultry processing plants. Financial support was provided by the Chicken Farmers of Saskatchewan (Saskatchewan Chicken Industry Development Fund), the Saskatchewan Agriculture Development Fund, and the Natural Sciences and Engineering Research Council of Canada.

References


