Fowl adenovirus Group I as a causal agent of inclusion body hepatitis/hydropericardium syndrome (IBH/HPS) outbreak in brazilian broiler flocks

Elena Mettifogo, Luis F.N. Nuñez, Silvana H. Santander Parra, Claudete S. Astolfi-Ferreira and Antonio J. Piantino Ferreira

ABSTRACT.- Mettifogo E., Nuñez L.F.N., Santander Parra S.H., Astolfi-Ferreira C.S. & Ferreira A.J.P. 2014. Fowl adenovirus Group I as a causal agent of inclusion body hepatitis/hydropericardium syndrome (IBH/HPS) outbreak in brazilian broiler flocks. Pesquisa Veterinária Brasileira 34(8):733-737. Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva 87, São Paulo, SP 05508-270, Brazil. E-mail: ajpferr@usp.br

Commercial broiler flocks from a farm located in the State of São Paulo, Brazil, presented diarrhea, depression, increased mortality and poor weight gain. Upon post-mortem examination, classical signs of Inclusion Body Hepatitis/Hydropericardium Syndrome (IBH/HPS) were observed, including enlarged pale yellow-colored livers and straw-colored liquid in the pericardial sac. In addition, gross lesions were also observed in the kidneys, pancreas, thymus, intestines and gallbladder. Samples of these organs were analyzed by PCR for the detection of the hexon gene of the Fowl Adenovirus (FAdVs) Group I. The results were positive for both flocks (A and B) assayed by PCR. The macroscopic lesions associated with the detection of FAdV Group I by PCR in several of these affected organs allowed for the identification of IBH/HPS. In fact, this is the first report in Brazil of IBH/HPS in broilers, which identifies FAdVs group I as a causal agent of the disease. These findings may contribute to the worldwide epidemiology of the adenovirus-mediated hepatitis/hydropericardium syndrome.

INDEX TERMS: Fowl adenovirus, hepatitis, hydropericardium, IBH/HPS syndrome, chicken.

INTRODUCTION
Fowl Adenovirus (FAdVs) expression appears to be ubiquitous in domesticated fowl and is often isolated from asymptomatic chickens (McFerran & Adair 2003, Wang...
This paper describes the differential diagnosis of IBH/HBS group 1 as a causative agent of the disease. Furthermore, zilian broiler chickens flocks, identifying avian adenovirus Hepatitis/Hydropericardium Syndrome (IBH/HPS) in Brazil, the first detection of Fowl adenovirus group 1 (chicken) was demonstrated by antibody detection with immunodiffusion tests for the presence of Avian adenovirus has been isolated from birds. Avian adenoviruses are further subdivided into three serological groups (I - III). The Fowl Adenoviruses (FAdVs) belong to Group I, which comprises five species (A to E) and 12 serotypes (1 to 12) and share a common group antigen with viruses isolated from chickens, geese, ducks and turkeys.

In Brazil, the first detection of Fowl adenovirus group 1 (chicken) was demonstrated by antibody detection with immunodiffusion tests for the presence of Avian adenovirus group 1. Although Fowl adenovirus group 1 was present in 78.2% of serum collected, its expression was not related to any disease. Adenoviruses belong to the family Adenoviridae. The genus Mastadenovirus contains adenoviruses that infect mammals, whereas the genus Aviadenovirus has been isolated from birds. Avian adenoviruses are further subdivided into three serological groups (I - III). The Fowl Adenoviruses (FAdVs) belong to Group I, which comprises five species (A to E) and 12 serotypes (1 to 12) and share a common group antigen with viruses isolated from chickens, geese, ducks and turkeys.

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A commercial poultry company’s flock of broilers had a continuous history of malabsorption syndrome, with poor weight gain, high feed conversion rates and diarrhea. The onset of these signs began the fourth week of bird life, and signs were more pronounced in flocks that were housed during months with higher temperatures. The farm had 21 flocks, with a population of 22,000 to 41,000 birds per flock and a density of 14.6 to 15.5 birds/m². Immunization procedures were routinely performed in the hatchery against Infectious Bronchitis Virus (IBV-Bio-Bronk-Vet H120), Infectious Bursal Disease Virus (IBDV-Gumbor-Mark-Vet), Marek’s disease (HVT-Bio Mark-Vet L) and fowl pox virus (FPV-Bouba Suave). All vaccines were obtained from the Bio Vet Laboratory, Vargem Grande Paulista, SP, Brazil. According to veterinarian reports, some attempts at antibiotic treatments were performed at the onset of signs, including bacitracin at 300 ppm/ton of feed for five days as a preventive treatment. This procedure was repeated 10 days later, with no significant improvement was observed.

On 13 March, 2011, two flocks (A and B) were chosen for analysis based on case history, clinical signs, necropsy and laboratory analysis. In the two flocks, the broilers were 41 days old and were at the end of their life cycle. The main clinical signs presented by the broilers were severe diarrhea, prostration, ruffled feathers, depigmentation of the leg skin, severe dermatitis, increased mortality after the fourth week of life, high feed conversion (1:2.10) and low weight gain. Poultry litter was very wet as a result of severe diarrhea. In flock A, the mortality rate reached 5%, and the final weight loss was 37%; in flock B, mortality was 2.4%, and the weight gain was 45%, which was lower than expected.

Virus detection by PCR technique

Ten chickens (five birds/flock) were sacrificed for postmortem examination. Fragments of the organs with or without gross lesions were collected and frozen at ~ 20°C for PCR to detect the Fowl Adenovirus (FAdVs) Group I hexon gene, and for differential diagnosis for Infectious Bronchitis virus (IBV), avian reovirus, Infectious Bursal Diseases virus (IBDV), and Chicken Anemia virus (CAV) according to the authors in Table 1. All samples were tested in duplicate, since nucleic acid extraction until the final PCR process.

Table 1. Primers sets, nucleotide sequences, amplicon sizes and the corresponding references that were used to screen for the viruses

<table>
<thead>
<tr>
<th>Virus detected</th>
<th>Primers</th>
<th>Nucleotide sequence (5' - 3')</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian Adenovirus group 1</td>
<td>hexon A</td>
<td>CAA RTT CAG RCA GAC GT</td>
<td>897</td>
<td>Meulemans et al. 2001</td>
</tr>
<tr>
<td>Infectious Bronchitis Virus</td>
<td>hexon B</td>
<td>TAGTGA TGM CCS GAC ATC AT</td>
<td>179</td>
<td>Cavanagh et al. 2002</td>
</tr>
<tr>
<td></td>
<td>UTR 41+</td>
<td>AGTC TCT ATC GCC AGG GAA ATG TC C</td>
<td>1120</td>
<td>Pantin-Jackwood et al. 2008</td>
</tr>
<tr>
<td></td>
<td>UTR 31</td>
<td>GCC GGT CCA ACT GCT GTA CCC C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UTR 11</td>
<td>GCT CTA ACT CTA TAC TAG CCA C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avian Reovirus</td>
<td>S4-F13</td>
<td>TAG GCC ATC CTA GCT GCA</td>
<td>631</td>
<td>Yamaguchi et al. 1996</td>
</tr>
<tr>
<td></td>
<td>S4-R1133</td>
<td>ATG ACA AAC GCC GTG ACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious Bursal Diseases Virus</td>
<td>VP2F</td>
<td>ACC ATA AAC GCC GTG ACC</td>
<td>675</td>
<td>Todd et al. 1992</td>
</tr>
<tr>
<td></td>
<td>VP2R</td>
<td>CGG TGG ATC GCT ACT GT A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken Anemia Virus</td>
<td>CAV4a</td>
<td>GAC TGT AAG ATG AGA AGA CCA C</td>
<td>734</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CAV4b</td>
<td>GCC TGA AGG CCT CAT TC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Table 3. ELISA results for Chicken Anemia Virus, Reovirus, Infectious Bronchitis Virus and Infectious Bursal Disease Virus and the average antibody titers of positive samples from the A and B flocks

<table>
<thead>
<tr>
<th>Agent</th>
<th>Flock A +/n</th>
<th>Positive samples</th>
<th>Flock B +/n</th>
<th>Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Anemia Virus</td>
<td>5/10</td>
<td>0.229 0.826</td>
<td>2/10</td>
<td>0.229 0.826</td>
</tr>
<tr>
<td>Reovirus</td>
<td>5/10</td>
<td>0.229 0.826</td>
<td>2/10</td>
<td>0.229 0.826</td>
</tr>
<tr>
<td>Infectious Bursal Disease Virus</td>
<td>5/10</td>
<td>0.229 0.826</td>
<td>2/10</td>
<td>0.229 0.826</td>
</tr>
</tbody>
</table>

DISCUSSION

Inclusion body hepatitis (IBH) and Hydropericardium syndrome (HPS) have been described as entities characterized by hepatic (swelling, discoloration, ecchymosis or petechial hemorrhages) and renal dysfunction (kidney inflammation, distension of the tubules and degenerative changes). These entities are characterized by basophilic inclusion bodies or eosinophilic hepatocytes and have been associated mainly with the group of adenoviruses belonging to serotypes 4, 8, 11 and 12 (McFerran & Adair 2003, Alemnesh et al. 2010). Although classical lesions in the heart and liver are characteristic of HPS, FAdVs have also been reported to cause lesions in other organs such as the pancreas, proventriculus, and lymphoid organs and in the respiratory system. Dama-

Fig.1. Broiler chicken with hydropericardium and hepatitis. The liver is pale, enlarged and discolored.

Fig.2. Heart of affected broiler. Characteristic straw-yellow liquid is visible in the pericardial sac.
ge to these organs caused by adenoviruses has been described previously, both alone and in combination with other viruses (Cowen et al. 1978).

In this report, atrophy of lymphoid organs (thymus and bursa) was observed in both flocks (A and B) and was associated with both FAdVs group I and CAV. Similar pathological lesions in lymphoid organs have been described in broilers with CAV. These lesions include thymic, splenic and bursal atrophy, aplastic bone marrow and anemia, which may vary in severity depending on the presence of other pathogens (Adair 2000). However, the increased intensity of gross lesions in flock “A”, which had lower antibody titers and was negative for CAV as assessed by PCR, suggests that the detected serotype of FAdV is capable of inducing the macroscopic lesions observed in these lymphoid organs.

Based on the available information on the vaccination programs performed at the hatchery against IBV and IBVD and the results obtained here, the lower antibody titers against the diseases observed in flocks A and B indicated that these antibodies were induced by vaccination and not by field virus infection. By contrast, the antibody titers against reovirus indicate a field virus infection because the flocks were not vaccinated against this disease. However, PCR analysis revealed that the organs were negative for reovirus. Although reovirus has been reported to cause injuries similar to those caused by FAdV (Ni & Kemp 1995), this virus causes tenosynovitis and consequently was not considered to be one of the possible causative agents of the signs observed in the chickens in this report.

CAV antibodies were higher in flock B, in which the signs and gross lesions were less intense than those observed in flock A. Furthermore, the PCR results indicated that this flock was negative for CAV. It has long been believed that adenoviral IBH results only when birds are also infected with IBDV, CAV, or other immunocompromising agents (McFerran & Adair 2003). In the 1970s, researchers were able to reproduce the disease using isolates from infected birds presenting lesions in the lymphoid tissue (McCracken et al. 1976, Grimes et al. 1977). Outbreaks of IBH in which no immunosuppressive pathogens were detected have been reported in Northern Ireland, Australia, Korea and New Zealand - countries that have experienced epidemic outbreaks of IBH in the absence of IBDV (Reece et al. 1986, Christensen & Saiuddin 1989, Choi et al. 2012). Experiments performed by Toro and colleagues could not confirm the hypothesis that the association of CAV and FAdVs group I is necessary for the induction of IBH/HPS syndrome (Toro et al. 2001). Those authors followed the premise that certain isolates, such as FAdV-4, can reproduce the syndrome by themselves, but other strains appear to require the presence of an immunosuppressive agent.

According to our results, the hepatitis and hydropericardium observed in the broilers were caused mainly by FAdV Group I. The presence of CAV according to ELISA and its accompanying immunosuppressive effects most likely caused a synergistic effect with FAdV that may have accentuated the general signs. To our knowledge, this is the first report of FAdV infection causing hepatitis and hydropericardium syndrome in Brazil. Further investigation is needed to verify the spread of this virus in Brazilian poultry flocks.

**CONCLUSION**

This is the first report of Inclusion body hepatitis/hydropericardium syndrome (IBH/HPS) caused by Fowl Adenovirus group I in Brazil. Indeed, this description may contribute to the worldwide epidemiology of adenovirus-mediated hepatitis/hydropericardium syndrome.

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**REFERENCES**


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**Table 4. PCR results for the detection of Fowl Adenovirus (FAdV), Chicken Anemia Virus (CAV), Reovirus, Infectious Bronchitis Virus (IBV), and Infectious Bursal Disease Virus (IBDV) from samples of several organs of chickens from flocks A and B**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Flock A</th>
<th>Flock B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thymus</td>
<td>Liver</td>
</tr>
<tr>
<td>FAdV</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CAV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reovirus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IBV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IBDV</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
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