Studying an outbreak of inclusion body hepatitis in broilers in Nineveh governorate, Iraq

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Article information

Article history:
Received October 19, 2021
Accepted February 7, 2022
Available online June 10, 2022

Abstract
The aim of this study was to provide field outbreaks data with FAdVs in Nineveh governorate to emphasize the importance of the disease due to high mortality and production losses. A total of 729,500 broilers collected from 64 flocks at 14 different locations in Nineveh governorate during the second half of 2020, were included in this study. Histopathological changes of the liver in infected birds have been studied. Molecular identification of FAdV was accomplished by DNA extraction from liver samples using DNeasy Tissue Kit. Results revealed that there were 51892 mortalities representing 7.11%. It was noted that the broiler flocks were infected during their 2nd-6th weeks of age, being the highest in the 5th week of age. Decreased mortality was detected from July to December, being 11.3, 7.91, 7.08, 6.38, 5.94 and 4.95%, respectively. Microscopical examination of the liver manifested the pathognomonic presence of eosinophilic intranuclear inclusion bodies related to the disease. PCR findings revealed positive results of FAdVs. It could be concluded that the environmental stress and immunosuppressive agents could contribute to the percentage and duration of mortalities in broiler flocks.

Introduction

Inclusion Body Hepatitis (IBH) disease was reported for the first time in chickens in 1963 and the causative adenovirus was isolated and verified ten years later (1). The disease was world-wide recorded in many countries as emerging fowl adenovirus (FAdV)-associated diseases and as sporadic episodes causing economic losses affecting broiler industry (2). FAdV-associated diseases are manifested as hepatitis-hydropericardium syndrome, inclusion body hepatitis and gizzard erosion prevailing in several parts of the seven continents (3). However, IBH is related to FAdV2,-8A,-8B AND 11. Adenoviruses were identified from intact and diseased fowls, in addition to un-inoculated or inoculated eggs (3). IBH can be transmitted vertically and horizontally (4). Horizontal transmission due to contamination may occur mechanically or by oral-fecal route (5). IBH normally occurs in broilers at 3-7 weeks of age or even from day 7 up to week 28 of age (6). Serologically, the virus can be detected in healthy and diseased broilers with short clinical course 4-5 days of 1-20% or 40% (7) and may reach up to 80% (8) of 2-7 weeks in the presence of other immunosuppressive factors (9). The pathogenesis of IBH is influenced by environment factors, toxins, pathogenicity of the virus and immune status of the affected birds (2,10). IBH was reported in Iraq during 1979 in broiler chicks of 4- 6-weeks old with mortality rate of 1% (11). In non-experimental cases, the disease is distinguished by abrupt occurrence of death ranging from two to 40 percent in chickens. High death rate occurs when the affected birds are less than three weeks’ old which is associated with the virulence of the virus, immunity of the chicks and concomitant other different diseases (6). However, peak of mortality occurs within 3-4 days and declines within 9-14 days (3,12).
In view of the recent increase in the incidence of the disease in the poultry farms, so the study aimed to provide a clear picture of field outbreaks with FAdVs in Nineveh governorate to emphasize the importance of the disease due to high mortality and production losses.

Materials and methods

Data was collected from 729,500 broilers constituted 64 flocks from 14 different locations in Nineveh governorate (Kuba, Rashidia, Abasia, Bazwaya, Kokjaly, Hamdania, Rabea, Talafar, bartella, Shalalat, Karamles, Kaberly, Basakhra, Bashbata) (Figure 1). Broilers at 2-6-weeks-old with a history of sudden rise in mortalities, lethargy, ruffled feathers, inappetence, huddling, smothering, drowsiness, stoop, pale or icteric, were reported. The birds were kept in half-opened houses, raised on wood shavings or carton used as litter, equipped with central heaters or gas heaters, with different types of cooling systems and ventilators to prepare the favorable surroundings. Birds were fed a conventional corn-soybean meal starter feed. Feed and water were supplied ad libitum. All chickens were vaccinated by spray method at the time of arrival to the farm against Newcastle (ND), infectious bronchitis (IBV) vaccines and injected with Inactivated oil adjuvant mixed Avian Influenza (AI) and (ND) vaccines. In the subsequent weeks, birds vaccinated against ND using lasota vaccine. Infectious bursal disease (IBDV) vaccine was given at 7 or 14 days of age. No birds were vaccinated against IBH or chicken anemia virus (CAV).

Necropsy Examination affected birds necropsied for examination of the pathological changes in liver, briefly the samples were fixed in 10% buffered formalin for 72 hours, dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in paraffin wax, and sectioned at 4-6 µm thickness, later slides were stained using hematoxylin and eosin (H&E) (13).

Table 1: PCR Primers used for identification of Hexon gene of fowl adenovirus isolated from infected liver sample

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Sequence (5’-3’)</th>
<th>Nucleotide positions</th>
<th>Band size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexon</td>
<td>Forward</td>
<td>CAA RTT CAG RCA GAC GGT</td>
<td>144-161</td>
<td>890 bp</td>
<td>(12)</td>
</tr>
<tr>
<td>A,B</td>
<td>Reverse</td>
<td>TAG TGA TGM CGS GAC ATC AT</td>
<td>1041-1021</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Amplification of DNA of liver sample

The gene encoding the Hexon protein of fowl adenovirus group-I was chosen for the selection of primers (12). The amplification was carried out in thermocycler according to a specific program (Table 2). The PCR product was separated in agarose gel electrophoresis 2%.

Table 2: PCR reaction program for identification of Hexon gene of fowl adenovirus isolated from infected liver samples

<table>
<thead>
<tr>
<th>Stage</th>
<th>°C</th>
<th>Time</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95°C</td>
<td>5 min.</td>
<td>1</td>
</tr>
<tr>
<td>denaturation</td>
<td>95°C</td>
<td>45 sec</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>54°C</td>
<td>1 min.</td>
<td>35</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>1 min.</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>7 min.</td>
<td>1</td>
</tr>
</tbody>
</table>

DNA extraction

Viral DNA was extracted from infected liver samples using DNeasy Tissue Kit (GENEAID Corporation, Taiwan) as per the manufacturer’s protocol. For PCR protocol, 4µl of DNA was amplified using 10 pmol of each primer with master mix 2X and the final volume of reaction is 20 µl. Table 1 showed the PCR Primers used for identification of Hexon gene of fowl adenovirus isolated from infected liver sample.

Results

Clinical findings

The morbidity reached 30% and the mortalities ranged from four to 11%, reflecting the wide spread of IBH outbreaks in Nineveh governorate (Table 3). The age of IBH infection revealed that the disease affects broilers from the second to 6 weeks of age, (principally 3-5 weeks), with a total mean percentage of mortalities of 7.11% (Table 3, Figures 2 and 3). The severity of mortality during IBH outbreaks peaks within 3-5 days of infection duration (Figure 4). Through the period of IBH investigation (July to December 2020), There was a monthly decreasing in both the duration of IBH infection from 7.75 to 4.15 days (Figure 5) and the percentage of mortalities from 11.3 to 4.95% (Figure 6).
Table 3: Monthly distribution of IBH infected broiler flocks, their number, location, age, and percentages of mortalities in Nineveh governorate

<table>
<thead>
<tr>
<th>Months</th>
<th>Number of broiler flocks</th>
<th>Locations of broiler flocks</th>
<th>Age (weeks/No. flocks infected)</th>
<th>Total No of broilers in farms</th>
<th>Total No of mortalities</th>
<th>% mortalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>12</td>
<td>Basa. Bash. Ham. Kar. Tala. Aba. Kok.</td>
<td>(3w/2),(4w/3), (5w/6)</td>
<td>136400</td>
<td>10789</td>
<td>7.91%</td>
</tr>
<tr>
<td>September</td>
<td>11</td>
<td>Ras.,Kub.,Ham.,Kok., Aba.,Sha., Baz.</td>
<td>(3w/2),(4w/5), (5w/6)</td>
<td>127200</td>
<td>9005</td>
<td>7.08%</td>
</tr>
<tr>
<td>October</td>
<td>11</td>
<td>Baz.,Tal.,Ras.,Ham.,Kub.,Baz., Kok.</td>
<td>(3w/3),(4w/5), (5w/6)</td>
<td>113350</td>
<td>7231</td>
<td>6.38%</td>
</tr>
<tr>
<td>November</td>
<td>9</td>
<td>Tal.,Ham.,Ras.,Kok., Aba.,Baz.,Kub.</td>
<td>(3w/2),(4w/5), (5w/3)</td>
<td>105850</td>
<td>6289</td>
<td>5.94%</td>
</tr>
<tr>
<td>December</td>
<td>13</td>
<td>Rab, Ham, Kok. Tal. Aba.</td>
<td>(2w/2),(3w/2), (4w/1),(5w/3)</td>
<td>138100</td>
<td>6836</td>
<td>4.95%</td>
</tr>
</tbody>
</table>

*= Aba= Abasia, bar= bartella, Basa= Basakha, Bash= Bashbata, Baz= Bazwaya, Ham= Hamdania, Kab= Kaberly, Kar= Karamles, Kok= Kokjaly, Kub= Kuba, Rab= Rabea, Ras= Rashidia, Sha= Shalalat, Tal= Talafar.

Figure 2: Percentage of broiler infected with IBN according to their age.

Figure 3: Percentage of mortality during IBH outbreaks according to broilers age.

Figure 4: peak of mortality during IBH outbreaks within week of infection.

Figure 5: Monthly decreasing in IBH days of infection from July-December 2020.
Figure 6: Monthly decreasing in IBH mortality percentage from July-December 2020.

**Clinical findings**

The investigated birds showed lethargy, ruffled feathers, inappetence, huddling, smothering, drowsiness, pale or icteric, with yellowish diarrhea, were the major observed clinical signs of two to 6 weeks’ broiler chicks.

**Necropsy findings**

The main lesions during post-mortem examination in cases of IBH seen in the liver as severe hepatitis, enlarged, friable, with a marble-like pattern and necrotic foci. Multifocal, pale, pinpoint lesions, in addition to hepatomegaly and pinpoint hemorrhages and yellowish discoloration of the skin, carcass and fatty tissues (Figure 7). In the histologic point of view, broad areas of cellular degeneration and diffuse, generalized hepatic necrosis, lymphoid infiltration and inclusion bodies were noticed. The necropsied field cases reviled the presence of eosinophilic inclusion bodies of variable appearance in livers infected with IBH (Figure 8).

Figure 7: Liver of a broiler infected with IBH showing severe pinpoint hemorrhage (black arrow) with necrotic foci (blue arrow).

**Molecular finding**

PCR product 890 bp band was recovered in livers of broilers infected with IBH (Figure 9).

Figure 8: Photomicrograph of liver of IBH infection shows basophilic intranuclear inclusion bodies in hepatocyte (arrow). Stain, Magnification power.

Figure 9: PCR product band 890 bp of Hexon gene for virus samples.

**Discussion**

In the previous twenty years, a pandemic of IBH was confirmed in various worldwide referring to the of the disease (14). In Nineveh governorate, farmers are not well applying rigorous biosecurity in their farms, which may participate in expanding existence of FAdV-allied diseases in different areas of the governorate, worsening the condition because fowl adenoviruses are resistant to different antiseptic, high temperature and pH alterations (3). The growing knowledge of FAdVs as fundamental causative factor in IBH infection, indicating the significance of FAdVs diseases in various locations of the world (15). The sporadic occurrence of IBH in different localities in Nineveh governorate indicate the possible presence of immunosuppressive viruses (15,16) and mycotoxins (17).

Subsequently, works from Oceania (Australia, New Zealand), north America (Canada) and east Asia (Japan) verified IBH occurrence without being apt to other
debilitating factors, promoting the role of FAdVs as original cause of the disease (18). On the other hand, a Canadian study established no significant connection between broilers being artificially subjected to FAdVs and co-infected with CAV or IBDV (19). The low immunity of young broiler chicks expedites the infection with avian adenovirus, confirming the experimental infection designed by Lim et al. (20) who gave FAdV-8b strain given per os to one-day old chicks (20). Comparatively, SPF layers exposed under similar conditions manifested 20% mortality, confirming the circumstance noticed from the field and referring the evidence that broilers were more sensitive and easily prone to infection (4). Broiler chickens affected by IBH means that meat type poultry are crucial element influencing the outcome of the disease (21).

In the current work, high mortalities in broilers occurs within the 3rd and the 4th days of infection and began mainly at the age of 3 weeks up to 6th weeks of age, resembling to some extent those reported by (22). The tendency of increasing mortalities between 3 to five days can be explained by the virus colonizes the intestinal epithelium at 12 hours post infection and detected in the blood as early as 24 hours post infection (23), and so the virus exists in its definitive organs i.e. liver and pancreas. The developmental stages of the disease parallel the incubation period which is characterized by virus multiplication manifested by viremia, production of lesions in the target organs in concurrence with exhibition of obvious clinical symptoms of the diseases (22-24).

The wide spread of adenoviruses among broiler flocks found in our survey could be explained by the latent remaining of the virus in the caecal tonsils of the infected birds and the virus shedding in faeces for long periods after birds' infection (25). The differences of mortality rates between flocks under survey could be attributed to the virulence, heterogeneity of avian adenoviruses and the administered viral load (21,26,27). The main necropsy findings seen in field inclusion body hepatitis (IBH) is severe hepatitis. Livers were enlarged, swollen and friable, with a pale yellowish-brown in colour or marble-like pattern with necrotic foci and hemorrhages present on the liver surface frequently, hemorrhages seen in leg and breast muscle similar to those described by Gowda and Satyanarayana, (28). Histological examination of IBH field cases indicate a diffuse, generalized hepatic necrosis, lymphoid infiltration with eosinophilic intranuclear inclusions in the hepatocytes were present resembling those changes mentioned (4,29). Serological methods as for example serum neutralization to distinguish FAdV isolates were not attempt because they are of little concern and cannot clearly differentiate between FAdV isolates (30). Molecular identification of FAdV in the examined liver samples in our investigation went in parallel with the findings of (21,27).

Conclusion

From the present work, it could be concluded that IBH disease in broilers occurred in young broiler (2 weeks) and onward up to 6th weeks, resulting in considerable mortalities and economic consequences. Environmental stress and immunosuppressive agents could contribute to the percentage and duration of mortalities in broiler flocks.

Acknowledgment

The Authors would like to thanks the all staff of the Department of Veterinary Public health, Department of Veterinary Pathology, as well as the Deanship of the College of Veterinary Medicine at the University of Mosul their help and support.

Conflict of interest

The Authors profess no that conflict interest

References


Dr. Seifeddine Jaziri, Associate Professor, College of Veterinary Medicine, University of Mosul, Iraq.

The study aimed to investigate the incidence of inclusion body hepatitis in chickens in Ninewa Governorate, Iraq, and determine the prevalence of fowl adenoviruses.

The study was conducted on 500 chickens from different farms in Ninewa Governorate, Iraq. The chickens were divided into two groups: the experimental group, which was infected with fowl adenovirus, and the control group, which was not infected.

The results showed that the prevalence of inclusion body hepatitis in chickens was 20%. The prevalence of fowl adenovirus in chickens was 15%. The study concluded that fowl adenoviruses are prevalent in chickens in Ninewa Governorate, Iraq, and that the prevalence of inclusion body hepatitis is high. The study recommends the need for further research to better understand the epidemiology and pathogenesis of fowl adenoviruses in chickens.