Short communication

Investigation of duck plague virus in hoar areas of Bangladesh

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Abstract

Duck plague (DP) is the most important infectious disease of geese, ducks and free-ranging water birds. The present study was conducted to determine the prevalence of duck plague virus followed by isolation and identification. For these purposes, a total of 155 cloacal swabs samples were collected randomly from duck of different hoar areas of Bangladesh including 45 (41 surveillance and 4 clinical) samples from Netrokona; 42 (40 surveillance and 2 clinical) samples from Kishoregonj; 30 samples from Brahmanbaria and 38 samples from Sunamganj. The samples were processed and pooled (1:5 ratio) for initial screening of target polymerase gene of duck plague virus by polymerase chain reaction (PCR) method. All the samples of a positive pool were then tested individually for identifying the individual positive samples. The result showed that out of 155 samples, 41 (26.45%) were found positive in which 17 were from Netrokona, where 15 (36.58%) were from surveillance samples and 2 (50%) were from clinical sample; 16 were from Kishoregonj, where 14 (35%) were from surveillance samples and 2 (100%) were from clinical sample; 2 (6.6%) were from Brahmanbaria and 5 (13.15%) were from Sunamganj. These positive samples were inoculated into 9-10 days embryonated duck eggs (EDE) through chorioallantoic membrane (CAM) route for the isolation of virus. The EDE died earlier was also chilled, and in a similar way, the CAMs were collected and again performed PCR for identification of virus. Out of 41 PCR positive samples, 26 samples were isolated and reconfirmed by PCR. Subsequently, DPV was isolated in primary duck embryo fibroblasts cell culture and confirmed by observing cytopathic effect (CPE).

(Key words: Duck Plague Virus, Isolation, PCR, Cell culture)


Introduction

Duck plague (DP) is an acute, sometimes chronic, contagious viral disease that occurs naturally only in ducks, geese and swans, all members of the family Anatidae of the order Anseriformes. Duck plague is caused by duck herpesvirus 1 (anatid herpesvirus 1) is a member of the Alphaherpesvirinae sub-family

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of the family *Herpesviridae* (OIE, 2018). In domestic ducks and ducklings, DP has been reported in birds ranging from 7 days of age to mature breeders. In domestic ducks the incubation period ranges from 3–7 days. Mortality usually occurs 1-5 days after the onset of clinical signs and is often more severe in susceptible adult breeder ducks. Most infected ducks that show symptoms eventually die; morbidity and mortality may reach 90-100 percent (Campagnolo *et al.*, 2001, Carter *et al.*, 2006). Recovered birds may be latently infected carriers and may shed the virus in the faeces or on the surface of eggs over a period of years (Richer and Horzinek, 1993; Shawky and Schat, 2002). So, it’s a great concern for the duck industry in the world including Bangladesh. In Bangladesh duck population is about 57.752 million (DLS, 2019; Ali, 2018). Major portion of the ducks are reared in semi intensive methods in the Haor areas of Bangladesh. Lands in these areas remain under water for 4-5 months of the year. These enormous water bodies in the areas are very convenient for duck rearing. Poor and ultra-poor people depend on duck as a sole source of their livelihoods (Bary *et al.*, 2018; Bhuiyan *et al.*, 2019; Ali and Hasan, 2018). In addition to these, ducks are the major source of duck meats and eggs for other areas of the country. However, there are several constraints of which infectious diseases are considered as the most leading causes of economic loss and discouraging duck rearing in this country (Das *et al.*, 2005; Ali *et al.*, 2019). The objectives of the study were to determine prevalence, isolation and molecular identification of Duck plague virus in selected areas of Bangladesh.

### Materials and Methods

#### Study areas

The four Haor areas including Mohanganj upazilla of Netrokona; Itna upazilla of Kishoregonj, Nasirnagar upazilla of Brahmanbaria and Taherpur upazilla of Sunamganj districts in Bangladesh were selected on the basis of the highest density duck population.

#### Collection of samples and processing

A total of 155 samples were collected from duck species of different haor areas of Bangladesh including 45 (41 surveillance and 4 clinical) samples from Mohanganj, Netrokona; 42 (40 surveillance and 2 clinicals samples from Itna, Kishoregonj; 30 samples from Nasirnagar, Brahmanbaria and 38 samples from Taherpur, Sunamganj. After collection, the samples were stored locally in veterinary hospitals at -20°C and then transported to the Virology Laboratory of the Animal Health Research Division, BLRI, Savar, Dhaka and stored at -80°C until tested. The samples were processed according to the protocol described by OIE Terrestrial Manual 2012 (OIE, 2018) and surveillance samples were pooled (1:5 ratio) for initial PCR screening of DPV. Samples from positive pools were then tested individually for identifying the individual positive samples.

#### Detection of duck plague virus

DNA was extracted from the processed samples by using QIAamp DNA Blood Mini Kit (Qiagen, USA) according to the manufacturer instructions. Then the extracted DNA was subjected to PCR for detection of duck plague virus targeting the *polymerase gene* (446 bp) by using specific primers described by OIE (2018) (Table 1). The
Duck plague (DP) is an acute, sometimes severe in susceptible adult breeder ducks. In domestic ducks the domestic ducks and ducklings, DP has been identified as the source of their livelihoods (Bary et al. 2006). Recovered birds collected separately from embryos and cloacal or tissue by PCR test were treated with antibiotic (2000 iu/ml Penicillin and 200 mcg/ml Streptomycin) and tested for sterility in fresh blood agar media at 37°C for 24h. Sterile inoculums were then inoculated into chorioallantoic membrane (CAM) of 9-10 day-old duck embryonated eggs using standard techniques (OIE, 2018). Each of the inoculated embryos was monitored daily for 6 days. After 6 days of post-infection (PI), all live EDEs were chilled overnight and CAM were collected separately from embryos and again performed PCR test for identification and confirmation of virus. Subsequently, isolation of DPV in primary duck embryo fibroblasts cell culture was done by observing cytopathic effect (CPE).

**Results and Discussion**

The prevalence results showed that out of 155 samples, 41 (26.45%) were found positive in which 17 were from Mohongonj, Netrokona, where 15 (36.58%) were from surveillance samples and 16 were from Itna, Kishoregonj, where 14 (35%) were from surveillance samples and 2 (100%) were from clinical sample; 2 (6.6%) were from Nasirnagar, Brahmanbaria and 5 (13.15%) were from Taherpur, Sunamganj (Table 2).

The surveillance samples also showed considerable presence of duck plague virus in the studied areas, which is almost similar to the findings of Ahamed et al. (2015), where they showed the overall prevalence rate of DPV was 18.1% (17/94) in three districts (Rajshahi, Mymensingh and Netrokona) of Bangladesh. Haque et al. (2006) revealed that the most important causes of duck mortality were duck plague. Duck plague together with duck cholera in Chatkhil Upazila in Bangladesh where the prevalence of these disease were 25.3% for both.

The positive samples were then inoculated into 9-10 days embryonated duck eggs (EDEs) through chorioallantoic membrane (CAM) route for isolation of the virus. After 6 days of post-infection (PI), all live EDEs were chilled overnight and CAM were collected separately from the embryos. The EDE that died earlier was also chilled, and in a similar way, the CAMs were collected (OIE, 2018) and again the virus was identified through PCR (Figure 1).

Out of 41 PCR positive samples, 26 isolated samples were confirmed by PCR. The

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
<th>Product size</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>5’-GAA-GGC-GGG-TAT-GTA-ATG-TA-3’</td>
<td>446 bp</td>
<td>OIE, 2018</td>
</tr>
<tr>
<td>Reverse</td>
<td>5’-CAA-GGC-TCT-ATT-CGG-TAA-TG-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
present isolation results were also similar to the results found by Hansen et al. (2000), Wallace et al. (2000), Ahmed et al. (2015) and Campagnolo et al. (2001). Subsequently, isolation of DPV in primary duck embryo fibroblasts cell culture was done by observing cytopathic effect (Figure 2). CPE is characterized by the appearance of rounded clumped cells that enlarge and become necrotic 2–4 days later. Wang et al. (2013) found that a distinct CPE of duck embryonic fibroblast cells inoculated with samples was observed, whereas cells in the negative control group grew normally.

Further studies are warranted to investigate the epidemiology of DPV, in Bangladesh.

### Table 2. Prevalence of duck plague virus in selected Haor areas of Bangladesh

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>Sample type</th>
<th>Sample No.</th>
<th>PCR test result</th>
<th>Virus isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohanganj, Netrokona</td>
<td>Cloacal</td>
<td>41</td>
<td>15</td>
<td>36.58</td>
</tr>
<tr>
<td></td>
<td>Clinical</td>
<td>4</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Itna, Kishoreganj</td>
<td>Cloacal</td>
<td>40</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Clinical</td>
<td>2</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Nasirnagar, Brahmanbaria</td>
<td>Cloacal</td>
<td>30</td>
<td>2</td>
<td>6.6</td>
</tr>
<tr>
<td>Taherpur, Sunamganj</td>
<td>Cloacal</td>
<td>38</td>
<td>5</td>
<td>13.15</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>155</td>
<td>41</td>
<td>26.45</td>
</tr>
</tbody>
</table>

### Conclusion

This study indicates the high prevalence of duck plague virus among duck population in the Haor areas of Bangladesh. Therefore,
Duck plague (DP) is an acute, sometimes fatal infectious disease of ducks. It belongs to the sub-family Anatinae of the Avian Herpesviridae virus family. The virus is highly contagious and spreads rapidly among susceptible ducks, resulting in high mortality rates. Most infected ducks that show symptoms usually die within a week of the onset of clinical signs and is often more virulent in domestic ducks. Mortality usually occurs 1-5 days after the reach 90-100 percent (Campagnolo et al., 2001).

The study indicates the high prevalence of duck plague in the Haor areas, which is almost similar to the results found by Hansen et al. (2000), Ahmed et al. (2013) and Wallace (2000), where the prevalence of these diseases were 25.3% for both. According to the results of the study, it can be observed that the Haor area has the most important causes of duck mortality. Duck plague together with duck cholera in Chatkhil Upazila in greater Mymensingh district of Bangladesh were duck plague. Duck plague together with duck cholera in Chatkhil Upazila in greater Mymensingh district of Bangladesh.


OIE. 2018. Manual of diagnosis tests and vaccines for terrestrial animals; Chapter 2.3.7. Available at link: https://www.oie.int/ fileadmin/Home/eng/Health_standards/tahm/2.03.07_ DVE.pdf (Accessed at 4 August 2020)
Duck plague (DP) is an acute, sometimes chronic, contagious viral disease that occurs naturally only in ducks, geese and swans, all of the order Anseriformes. Duck plague is caused by duck herpesvirus, a member of the Alphaherpesvirinae subfamily, of the family Herpesviridae. The incubation period ranges from 3–7 days. Mortality usually occurs 1–5 days after the onset of clinical signs and is often more severe in susceptible adult breeder ducks.

Most infected ducks that show symptoms die within 1–5 days after the onset of clinical signs, but some survive longer. A total of 155 samples were collected from the Haor areas of Bangladesh where the prevalence of these diseases is very high. The samples were collected from Kishoregonj, Nasirnagar upazilla of Netrokona, Mohanganj, Brahmanbaria and Itna, Kishoregonj districts in Bangladesh.

The surveillance samples also showed positive in which 17 were from Mohongonj, Kishoregonj, where 14 (35%) were from surveillance samples and 2 (100%) were from clinical samples. The findings of Ahamed et al. (2006) revealed that DPV was 18.1% (17/94) in three districts out of 155 samples, 41 (26.45%) were found positive in which 17 were from Mohongonj, Kishoregonj, where 14 (35%) were from surveillance samples and 2 (100%) were from clinical samples.

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References


