Identification of potential risk factors for recurrent outbreaks of Infectious Bursal Disease (IBD) in poultry farms of Bogra District, Bangladesh in 2015

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Abstract: An outbreak investigation was conducted in poultry farms of two Upazilas (Bograsadar & Sherpur) of Bogra district of Bangladesh to identify potential risk factors associated with occurrence of Infectious Bursal Disease (IBD)/Gumboro during February-March 2015. A total of 75 farms were recruited under a cross-sectional study of which 40% (n=30) ‘infected farms’ and 60% (n=45) ‘non infected farms’ from the 2 Upazilas. The infected farms were included from active and passive surveillance data of two Upazila Livestock Offices and District Veterinary Hospital (DVH), Bogra. The non-infected farms were included by convenient sampling technique from the same areas of the two Upazila. The data were collected from the poultry farms using pretested semi-structured questionnaire. A total of 75 farmers were interviewed of which 87% (n=67) male farmers, 11% (n=8) female farmers with an average age of 38(±9) years. Sixty percent (n=45) farmers were rearing Sonali type of bird with a flock size 1554(±745) and 40% (n=30) farmers with broiler type of bird with a farm size 828(±263). Among the infected farms, 86.67% (n=26) farms were infected in Sadar Upazila and 13.37% (n=4) farms infected in Sherpur Upazila. The case fatality rate in Broiler and Sonali were 47.47%, 30.32% respectively with an average 33.26%. The study found that 26.67% (n=20) farms had practiced daily disinfection and 34.67% (n=26) farms did not allow visitors in the farms. The potential risk factors relating to biosecurity aspects were found significant in this study at level of p≤0.05, were, “not practicing all in all out”(OR= 11.15, 95% CI= 2.03-61.16, p=0.006), “not practicing cleaning and disinfection daily”(OR=7.92, 95% CI=1.2-52.28, p=0.03), “having partial netting of the farm” (OR= 36.97, 95% CI= 3.40-402.40, p=0.003) along with “inappropriate gap between two production cycle” (OR= 15.40, 95% CI= 2.27-99.57, p=0.005). The results from PE presented that good quality chicks (DOCs), farm bio-security, detection of antibody titer day 1 in conjunction with implementing proper vaccination schedule to be followed for successful IBD prevention and control at the farm level. In conclusion, proper bio-security measures with strict vaccination protocol should be maintained mandatorily to control of IBD at the poultry farm in this district.

Keywords: Infectious Bursal Disease (IBD); Gumboro; poultry; risk factors; Bangladesh

1. Introduction
In Bangladesh, the poultry sector emerged during 1980s has been taken its full shape now-a-days. About 7-8 million people directly and indirectly involve in this sector, of which 40 percent are women. At present, total investment in this sector amounting 25,000 million BDT (Equiv. 350.00 million USD) and that to be ascended up to 50,000 million BDT by 2020. This subsector is being kept as a second in position relating to woman empowerment next to Agriculture sector in the rural economy (WPSA, B, 2015). The country has 261.77 million chickens and 50.52 million ducks. The national consumption by year 2020 of poultry meat and chicken eggs is projected to be 307 thousand metric tons and 5.866 million respectively (anon/ unpublished data).
There are many economic important diseases in poultry in Bangladesh such as New Castle Disease (NCD), Infectious Bursal Disease (IBD) and Duck Plague (DP). Of which IBD is very important one, found almost all commercial poultry rearing districts of Bangladesh. The disease also present in most of the chicken producing countries in the globe. Infection can rarely be avoided, mostly due to the high resistance of its causal agent in the environment, and vaccination is considered the only way to prevent the negative consequences of the disease. Adopting a strategy of a sound vaccination with strict biosecurity measures could prevent the infection at the farm level (Fussel, 1998).

The causal agent of Infectious Bursal Disease (IBD) is a virus under a member of the genus *Avibirnavirus* of the family *Birnaviridae*. Since the disease may occur in wide range of avian species like turkeys, ducks, guinea fowl and ostriches, the clinical signs solely manifest in chickens. Only young chicken are clinically affected. The severe form of the acute disease found in the age of 3–6-week-old birds is related to high mortality rate. Moreover, a less acute or subclinical disease is common in 0–3-week-old chicken. Since the virus affects on the bursa of fabricius, it cause secondary problem later on. IBD virus (IBDV) causes lymphoid depletion of the bursa, and if this occurs in the first 2 weeks of life, significant reduction of the humoral antibody response may result. Two distinct serotypes of infectious bursal disease virus (IBD) are known to be present. Serotype 1 virus causes clinical disease in chickens younger than 10 weeks. Usually the older chickens show no clinical signs (OIE, 2008). Antibodies are sometimes found in other avian species, but no signs of infection are seen. Serotype 2 antibodies are very prevalent in turkeys and are occasionally found in chickens and ducks. There are no reports of clinical disease caused by infection with Serotype 2 virus (Lasher et al., 1994).

Gumboro one of the major threats of poultry farming in Bangladesh and there is none option to prevent IBD without vaccination (Lukert et al., 1997). However, Gumboro outbreaks also found in the vaccinated birds (Lukert et al. 1997, Hafez et al., 2002). In Bangladesh various vaccines against IBD are commercially available. Some vaccines were tested their protection level experimentally giving challenge with IBDV and both significant and insignificant increase of antibody titre were reported (Islam et al., 2005). Some commercially available vaccines became fail to give protection against IBD in a number of commercial poultry flocks. Different determinants are considered to be responsible for Gumboro vaccine failure. It is critical to vaccinate commercial chickens that have maternally antibodies at the optimum time (Tsukamoto et al., 1995). Vaccination during low maternally derived antibody titre shows better immune response than high maternal antibody titre (Giasuddin et al., 2003). Again, the immunogenicity of the virus may fluctuate between strain to strain (Rosales et al., 1989; Abdel-Alim et al., 2001) and the invasive vaccine strains are able to break through higher maternally derived antibody level (Kouwenhoven et al., 1994).

Vaccination against gumboro could be suggested after detecting maternal antibody in Day Old Chicks (DOCs) (Lukert et al., 1997). Three types of live gumboro vaccine available in the market are being practicing at the farm level are: mild, intermediate and intermediate plus. All types of vaccines administer through water or ocular route.

Gumboro (IBD) is a transmissible disease. The possible risk factors for incursion of Gumboro cases to be considered practicing all in all out, interval between two batches/ production cycle with proper cleaning and disinfection along with other biosecurity measures. The mortality may differ 10%-50% depends on virulent of the virus that could be reduced by early diagnosis as well as proper medication. It is a common problem nowadays in broiler and cockerel farms as continuous changing of batches than layer farms. The mortality in gumboroinfected birds reached highest at the day 3 and then it follows a down ward trends until day 6. The disease may occur some areas in the country in combination with LPAI and NCD.

Bogra is a district of northern part of Bangladesh. The district is considered as a poultry hub in the country especially for the meat chicken i.e. Sonali. This type of poultry was developed by cross-breeding between two exotic breeds, Faoumyi (hen) and Rhode Island Red(RIR, Cock) and adapted throughout the country especially in the northern districts of Bangladesh and considered to be moderately as native chicken in Bangladesh that supply major meat in the poultry supply chain of the country (FAO, 2015). The monthly collected secondary surveillance data demonstrated that the disease prevails in this district throughout the year.

The recurrent outbreak of Gumboro (IBD) in this district create further obstacle to fulfill the national demand of meat. Since there are a very limited study was conducted in this district to explore the possible risk factors responsible for the outbreaks, the present study will explore situation in this regards. That streamlines to underscore potential risk factors relating to IBD outbreaks in this district that could be addressed the actual disease burden.
2. Materials and Methods

2.1. Study area and study design

Bogra Sadar and Sherpur sub districts of Bogra District, Northern part of Bangladesh (Figure 1) were selected to conduct the investigation of IBD outbreaks in poultry during a period of February-March 2015.

A cross-sectional study was designed to find out the risk factors associated with IBD. The data on ‘suspect’ IBD infected farms recorded through passive surveillance of Upazila Veterinary Hospitals of the Upazila (sub-districts) and also from District Veterinary Hospital (DVH) were collected by our team. The confirmation of infected case farms were done solely by post mortem findings. Data from both ‘suspect case farm’ were considered as ‘cases’ for data analysis. The ‘non case’ farms were selected from the same Upazilas (Sub-districts) by convenient sampling technique.

2.2. Definition of case and control-farms

2.2.1. Infected case-farm

2.2.1.1. Suspected cases at the farm level

Poultry (Broiler, Sonali) that showed at least 2 clinical signs from the following -

The birds at farm level showing clinical signs anorexia, high fever, variable degrees of whitish diarrhea, depression, ruffled feather, huddling together, and finally deaths in Bogra Sadar (Arulia, Gokul, Sabgram, Shkerkola, Sagordighi) and Sherpur (Dorimokondo, Krishnapur) Sub districts of Bogra district during the periods in between February - March 2015.

2.2.1.2. Post mortem confirmation

Pin point haemorrhage in the thigh and breast muscles, enlarged and necrosed bursa of fabricius, yellowish gelatinous membrane over the swollen bursa, hemorrhage on the bursa, mucous containing drooping found in the ascending part of small intestine including above clinical signs.

2.2.2. Non case farms

The farm from the same geographical location as non-case farm by convenient sampling technique, consisting of birds not showing any of the above mentioned clinical signs during February-March 2015.

2.3. Data collection, management and statistical analysis

The data on demographics and risk factors were collected from both infected case and non case farms using pretested semi-structured questionnaires. The risk factors included in the questionnaires were: netting of the farm, exposure of previous outbreak, daily disinfection, adjacent to LBM, appropriate gap between two
production cycles, practicing all in all out along with entry allow in the farm. The data on demographics, risk factors and disease outcome were entered and validated in Microsoft Excel® worksheet and imported into Epiinfo 7 program (CDC, 2012) for statistical analysis. Multivariate logistic regression analysis was performed to evaluate associations of potential risk factors with the IBD outcome (case-farms versus non-case farms). A p-value of 0.05 was used to determine statistical significance.

2.4. Focus group interviews
Focus group discussions with poultry farmers were conducted using participatory epidemiological (PE) approach on IBD (Gumboro) in the villages. Topics such problems in IBD control, suggestion for improvement of vaccination procedure and Any Other Business (AOB) were discussed. Similar approach was undertaken with focus group discussions with livestock personnel (veterinarians/para-veterinarians). Data from both discussions were recorded in pre-formatted questionnaires. Proportions were calculated to describe the data and to prioritize the issues related to IBD.

3. Results
Of 75 famers 89% (n=67) famers were male and 11% (n=8) famers are female with an average age 38(±9) years. The training information relating to poultry farming has been as shown in Figure 2.

![Figure 2. Training of the farmers.](image)

Among the 75 poultry farms, 50% (n=37) farms adjacent to public habitat, 34 % (n=45) farms near to roadside and 5% (n=4) farms were near to Live-Bird Market (LBM).

Of 75 farms, 40% (n=30) was broiler and 60% (n=45) was Sonali (Cock) cock farms. The average flock size in broiler farm was 828 (±263) with a range of 500 to 1200 birds. In Sonali type poultry farm (Cock) the average flock size was 1554(±745) with a range of 500 to 5000 birds. The farm distribution on the basis of infectious status is shown in Table1.

<table>
<thead>
<tr>
<th>Type of bird</th>
<th>Sub-districts</th>
<th>Infected</th>
<th>Non-infected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Passive</td>
<td>Active</td>
<td>Total</td>
</tr>
<tr>
<td>Broiler</td>
<td>Sadar</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Sherpur</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sonali (Cockel)</td>
<td>Sadar</td>
<td>18</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Sherpur</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>2 sub-districts</td>
<td>26</td>
<td>4</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 1. Distribution of farms on the basis of infectious status in two Upazilas.

The average, minimum and maximum ages of broiler and Sonali (cockel) type of poultry were found during investigation as shown in Table 2.
Table 2. The average, minimum and maximum ages of broiler and Sonali (cockerel) type of poultry.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Broiler</th>
<th>Sonali (Cockerel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>23±4</td>
<td>24±9</td>
</tr>
<tr>
<td>Maximum</td>
<td>28</td>
<td>72</td>
</tr>
<tr>
<td>Minimum</td>
<td>18</td>
<td>3</td>
</tr>
</tbody>
</table>

3.1. Type, frequency of vaccination, age of the first vaccination, and antibiotic use during outbreak

Of 75 farmers, 100% farmers were being practicing Gumboro live vaccine twice or triple time in a same production cycle with live Gumboro hot/intermediate type vaccine where first vaccine introduced at the age of day 8±1. One hundred percent farmers were using antibiotics from Day 1 as preventive measures (wrong practice) for subsequent infection.

3.2. Bio-security measures

3.2.1. Incomplete netting

Eighty percent farms (n=60) used complete netting of the farms whereas 20% had partial netting. The use of partial netting showed significantly higher likelihood of IBD infection (OR=36.97 95% CI=3.40-402.40, p=0.003).

3.2.2. Daily cleaning and disinfection (C&D) practice

Twenty seven percent farms (n=20) were practicing daily cleaning and disinfection, where as 73% (n=55) did not practicing. “Not practicing daily cleaning and disinfection” showed significantly higher likelihood of IBD infection (OR=7.92 95% CI=1.02-52.28, p=0.03).

3.2.3. Entry allow in the farms

Sixty five percent (n=49) farms allowed entry in the poultry farm where as 34.67% (n=26) farms did not allow. No significant association was found between entry allow and the likelihood of IBD infection.

3.2.4. LBM adjacent

Ninety percent farms (n=70) farms were not located adjacent to Live-bird market and 6.67% (n=5) were located farms adjacent to LBM. No significant association was found between adjacent to the LBM and the likelihood of IBD infection.

3.2.5. Workers lives outside

Workers lived inside in 81% (n=61) farms and outside in 19% (n=11) farms. No significant association was found between workers lives outside and the likelihood of IBD infection.

Table 3. Result of multivariable logistic regression analysis between potential risk factors and IBD Infection (number of infected case-farms= 30, number of non-infected farms= 45).

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Number of farms</th>
<th>Adjusted Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposure factor</td>
<td>No Exposure to risk factor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>Non-case</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>Non-case</td>
<td></td>
</tr>
<tr>
<td>Having incomplete netting of the farm</td>
<td>12</td>
<td>3</td>
<td>36.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>(3.39-402.30)</td>
</tr>
<tr>
<td>Not practicing daily cleaning disinfection</td>
<td>27</td>
<td>28</td>
<td>7.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>(1.2-52.28)</td>
</tr>
<tr>
<td>Not following “all in all out”</td>
<td>25</td>
<td>15</td>
<td>11.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>(2.03-61.15)</td>
</tr>
<tr>
<td>Not allowing gap(at least 15 days) between two production cycle</td>
<td>24</td>
<td>16</td>
<td>15.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>(2.27-99.57)</td>
</tr>
<tr>
<td>History of previous outbreak within 1 year</td>
<td>22</td>
<td>17</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>(0.72-14.04)</td>
</tr>
<tr>
<td>Allow entry in the farm</td>
<td>11</td>
<td>14</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>(0.48-3.39)</td>
</tr>
<tr>
<td>LBM Adjacent to the farm</td>
<td>3</td>
<td>2</td>
<td>2.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27</td>
<td>(0.37-15.23)</td>
</tr>
<tr>
<td>Worker lives outside</td>
<td>27</td>
<td>34</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>(0.73-11.49)</td>
</tr>
</tbody>
</table>
3.3. Result from focus group interviews

The first focus group discussions of poultry rearing farmers of Sagordhigi village of Bogra Sadar Upazila consisted of 4 men and 1 woman representing 27-46 years of age. The occupation of the farmers was homemaker and poultry farming. Seventy-five percent farmers were rearing Sonali type of poultry specially for meat purpose and 25% broiler poultry. One hundred percent farmers immunized their birds against Gumboro using hot/intermediate type of vaccine.

A second focus group discussion consisted of 5 veterinarians and para-veterinarians from Bogra Sadar, Sherpur Upazila. The outputs of the focus groups discussion were recorded categorically and shown in Table 4 with regards to issues encountered in Gumboro prevention and control.

Table 4. Result of focus group discussion with farmers and veterinarians/para-veterinarians.

<table>
<thead>
<tr>
<th>Issues discussed by farmers</th>
<th>%</th>
<th>Issues discussed by Veterinarians/Para-veterinarians</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good quality chicks (DoC)</td>
<td>50</td>
<td>Farm biosecurity</td>
<td>40</td>
</tr>
<tr>
<td>Vaccine price more</td>
<td>30</td>
<td>Provision of antibody detection on Day 1 by the hatchery for proper timing of vaccination</td>
<td>30</td>
</tr>
<tr>
<td>Less training/Less knowledge</td>
<td>20</td>
<td>Cool Chain maintain</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proper vaccination(follow vaccination Schedule)</td>
<td>10</td>
</tr>
</tbody>
</table>

The focus groups opined on the issue of discriminatingly using antibiotics cause immune suppression enhance susceptibility to disease like IBD, the disease may occur in combination with Low Pathogenic Avian Influenza (LPAI)& NCD along with vaccination is not doing rightly. They opined that disease may prevalent more in Sonali poultry than broiler and layer.

A few suggestions that had been noted through the 3 focus groups discussions were: (i) Proper vaccination in parent stock (ii) Provision for detection of Gumboro antibody at the day-1 (iii) farm biosecurity could be bolstered to avert Gumboro infection (iv) maintain cool chain and proper vaccination schedule and (v) consult with vets for any type of drug use, even antibiotics.

4. Discussion

The results indicate that Sonali type poultry was more susceptible to Gumboro than broiler. The morbidity was less in broiler than the Sonali type poultry, but Case Fatality Rate (CFR) was higher in Broiler than Sonali type of poultry. The result is moderately compliance with result with the finding by Islam et al., 1998 as mortality as high as 70% in layers and 30% in broilers.

The study revealed that all the farmers introduced two/three time vaccination using intermediate/hot vaccine in the same production cycle. However, IBD outbreaks occurred as may be vaccination that relates to a) Vaccine type, storage, transportation and handling, b) Condition of the bird, and c) Administration of the vaccine (Hussain et al., 2003). It has been presumed that with emergence of new variants, the existing vaccines are no more effective to control the disease (Tariq, 1999).

It has been revealed that the exact timing of IBD vaccine administration in broiler chicks is crucial (Ather, 1993; van den Berg et al., 2000). As a result, the exact antibody titer is to be needed to comprehend for determining the age at which the chick will be susceptible to IBD (Lukert et al., 1997). Vaccination during low maternally derived antibody titre shows better immune response than high maternal antibody titre (Giasuddin et al., 2003). The farmers don’t know the antibody titre for 1st dose of vaccine inoculation. As the most of the farmers were using antibiotics from the Day 1, cause immune suppression and failure of vaccine (Tariq, 1999). However, as most of imported vaccine is not being used local isolates (Bhattacharjee et al., 1996), that could be considered as further bottleneck of immune response in poultry.

The farm bio-security is very important to delimit virus inclusion at the farm level. The potential risk factors/protective factors relating to biosecurity were found significant in this study are, not practicing all in all out (mixing age of farming), cleanliness and disinfection not daily implemented, incomplete (having partial netting) netting and not implementing proper gap in between two production cycle (at least 15 days).

The poor bio-security measures compel to survive Gumboro virus in the environment more than 4 months (Allan et al., 1982). Moreover the virus has the mutagenic capacity and become resistant to the environment.
Proper gap between two production cycles and appropriate C&D can prevent the Gumboro infection in the next batch. Complete netting (primary barrier) exclude animal and wild birds/scavenging bird to the farms as a carrier of infection. Daily C&D minimize microbial load in the farm. For successful Gumboro control, good quality of chicks, antibody detection at the Day 1, cool chain maintain, proper storage of vaccine, awareness creation among the farmers along with proper following of vaccination schedule would be more imperative.

However, other bio-security related risk factors, like exposure of previous outbreak within 1 year, live bird market adjacent to the farm, poultry workers lives outside of the farms were found insignificant in this study. The study has a few bias/limitations, such as: All areas of outbreaks were not covered in the study. The layer farms were excluded from the study as limited number present in this district. Proper sampling technique was not followed in the cross sectional study as time constraint. The study was solely relied on clinical sign and post mortem findings rather than confirmatory test (lab diagnosis).

5. Conclusions
Bio-security measures should be strictly maintained for successful prevention of IBD at the farm level, are, netting (Complete) of the farm, cleaning and disinfection (C&D) of farm every day. Proper gap at least 15 days should be allowed between two production cycles with appropriate C&D. All in all out should be mandatorily maintained. Vaccination in breeding flocks must be ensured for better antibody level in the DOC. Assaying of the antibody titre in DOC for fixing the timing the first dose of Gumboro vaccine should be ensured. Farmers should be followed a strict vaccination protocol. However, Veterinary Certification is necessary for using antibiotics and drugs. The local isolates could be used for production of vaccine. Good quality chicks should be ensured to prevent and control of all poultry diseases. Proper training is necessary to educate the farmers.

Conflict of interest
None to declare.

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