PATHOGENICITY OF IBDV RELATED TO OUTBREAKS IN THE VACCINATED FLOCKS AND THE CAUSES OF VACCINATION FAILURE

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ABSTRACT

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Pathogenicity of infectious bursal disease virus (IBDV) related to the outbreaks in the vaccinated flocks and the probable causes of vaccination failure were field based investigated during March, 2007 to March, 2008 at Dinajpur district of Bangladesh. The virus was pathologically determined as very virulent infectious bursal disease virus (vvIBDV) and the probable causes of vaccination failure were identified. Among the 23 Gumboro incidences in the vaccinated flocks, 15 in broiler, 3 in layer and 5 in cockerel flocks were recorded during the course of observations. The number of the birds in the farms was variable ranging from 250-1250 and they were reared on litter. The flocks were divided into five groups basically based on the severity of the disease and the mortality patterns. A relationship between the mortality rate and bursal lesion scores was determined. One apparently normal and another one unvaccinated affected flocks were also included in this study for the comparison. The birds were vaccinated with commercially available Gumboro vaccines containing intermediate strains. The clinical signs of the affected birds of the vaccinated flocks were noted during the physical visit of the farms and the farmer's complaints were also emphasized. The birds were examined systematically at necropsy and the lesions of the muscles and bursa of Fabricius were recorded. Only the bursae were collected, preserved at 10% formalin solution and processed for the histopathological study giving emphasis on bursal lesion scores. The pathogenicity of the virus related to vaccination failure was evaluated groupwise. The clinical signs of the affected birds were more or less similar to the signs generally developed due to the infection with vvIBDV, and clinically characterized as anorexia, high fever, whitish diarrhoea, dehydration, ruffled feathers, drowsiness and death. The morbidity of the affected flocks was around 100%, and the mortality rate was variable ranging from 7–38%. Highest mortality rate was recorded in cockerel (38%) followed by layer (27%) and broiler (7-19%), respectively. At necropsy, the birds were severely dehydrated and varying degrees of haemorrhages were found in thigh and breast muscles. The bursa of Fabricius was swollen, oedematous, haemorrhagic and atrophied containing cheesy exudates. Histopathologically, varying degrees of lymphoid depletion, necrosis, and reactive cells infiltration, cystic formation of the follicles with or without fibroplasia were seen in the bursae. Highest bursal lesion scores were recorded in the vaccinated flocks was more commonly similar to that of unvaccinated affected group. There was a proportional relationship between the pattern of mortality and the bursal lesion score in the respective group. The suspected causes of vaccination failure were also identified during the farm visit and from the farm records. The clinical and pathological findings with significantly high scores in the bursal lesions would suggest that outbreaks in the vaccinated flocks were undoubtedly vaccination failure, closely associated with different factors.

Keywords: Gumboro disease, vaccination failure, pathogenicity

INTRODUCTION

IBDV is highly infectious and very resistant to inactivation. There is none alternative without vaccination to prevent IBD or Gumboro disease (Lukert and Saif, 1997), but the outbreaks in the vaccinated flocks are also reported (Muhammad *et al.,* 1996; Hafez *et al.,* 2002). In order to control IBD with live vaccines, it is critical to vaccinate commercial chickens that have maternal antibodies at the optimum time. Live vaccines have the ability to overcome the maternal antibody at a certain level. Vaccination during low maternal antibody titre shows better immune response than high maternal antibody titre (Giasuddin *et al.*, 2003). But it is very much difficult to field based determine when maternal antibodies in chicks will decline to levels that vaccine can overcome as well as the optimum time of vaccination.

The apparent inability to control IBDV infections through vaccination sometimes may be due to improper administration of vaccine virus, antigenic differences among the viruses (Rosenberger *et al*., 1987), insufficient potency of the live attenuated vaccine virus (Ismail and Saif, 1991), interference between the residual maternally derived antibodies and the vaccine virus (Eterradossi, 2001). The vaccine prepared from classical strain did not give protection against variant IBDV strains (Snyder, 1990). Again, the immunogenicity of the virus may differ between strain to strain (Rosales *et al.,* 1989a, b, c; Abdel-Alim and Saif, 2001). The invasive vaccine strains are able to break through higher maternally derived antibody level (Kouwenhoven and van den Bos, 1994). The intermediate vaccine strain produced moderate to severe bursal lesions reported by many reseachers (Franciosini and Coletti, 2001). The better protection with more virulent strain of IBDV is due to more antigenic stimulation based on higher and longer replication in lymphoid tissues (Rautenschlein *et al.,* 2001). There is no evidence of antigenic variation between classical and vvIBDV strains and they belong to classical serotype I (van der Marel *et al.,* 1991). The

genetically engineered tissue culture adapted vvIBDV was attempted to use as vaccine candidate, but the attempt was not yet successful for its reversion (Raue *et al.,* 2004). This study was conducted for the field based evaluation of the pathogenicity of the viruses responsible for the outbreak in the vaccinated flocks and to investigate the probable causes of vaccination failure.

MATERIALS AND METHODS

Experimental chickens/Clinical cases

Gumboro outbreaks in the vaccinated flocks and the probable causes of vaccination failure were investigated at Dinajpur district of Bangladesh and the laboratory examinations were conducted at the Department of Pathology and Parasitology of Dinajpur Government Veterinary College, Dinajpur, Bangladesh.

Table 1. Grouping of the different flocks and their relative clinical history

Experimental group	No. of incidences	Age of bird when reported	Morbidity (%)	Mortality (%)	Birds examined at necropsy	Bursae examined at histopathology
Apparently normal	0	0	0	0	3-5 birds/flock	3-5/group
Broiler 1	3	14-28	Around 100	7	As above	As above
Broiler 2	7	14-28	Around 100	13	As above	As above
Broiler 3	5	14-28	Around 100	19	As above	As above
Layer	3	19-35	Around 100	27	As above	As above
Cockerel	5	18-29	Around 100	36	As above	As above
Unvaccinated infected group	4	17-26	Around 100	47	As above	As above

A total of 23 outbreaks in the vaccinated flocks among which 15 in broiler, 3 in layer and 5 in cockerel flocks were recorded during the course of observation. The number of the birds in the farms was variable ranging from 250 to 1250 and they were reared on litter. The age and population of the birds per flock, biosecurity of the farms, previous history of Gumboro outbreaks, intervals between the batches, rearing of one more batches in the same farm at the same time, etc. were also recorded.

Grouping of the flocks

The flocks were categorized into five groups basically based on the severity of the disease and the mortality patterns. The mortality rate was determined from the farm records.

Vaccines and vaccination

The birds were vaccinated with the commercially available Gumboro vaccines containing intermediate plus strain of IBDV. The vaccines and vaccination schedule, transportation, preservation, preparation and administration of vaccines were thoroughly investigated specifically to find out the clue(s) of vaccination failure.

Clinical findings

The clinical signs were recorded during the physical visit of the affected flocks and the farmer's complaints about the affected birds were also considered. One apparently normal and another one unvaccinated affected flocks were also included in this study for the comparisons.

Necropsy

The necropsy examination of the birds was done systematically as per standard procedure (Charlton, 2000). The general appearances of the birds and the visible gross morbid lesions of the muscles and the bursa of Fabricius were noted. The concurrent infections, if any were also investigated. Only the representative bursae were collected at 10% formalin solution.

Histopathology and bursal lesion score

A part of the each sample of the formalin fixed bursa of Fabricius was processed for paraffin embedding, sectioned and stained with haematoxylin and eosin as per standard procedures (Luna, 1968) for the histopathological examination under light microscope with variable magnifications. On histopathological examination, a bursal lesion score was determined on the basis of the following criteria (Raue, *et al.,* 2004): score 0 = apparently normal lymphoid follicles; score 1 = mild lymphoid depletion indicated by just thinning of the lymphocyte population without any sign of focal necrosis or remarkable oedema; score 2 = moderate lymphoid depletion along with focal

necrosis and interfollicular oedema; score 3 = severe lymphoid depletion virtually leaving no lymphocyte but only reticular cells and proliferating fibrous tissue; and score 4 = atrophy of follicles usually with cystic spaces, infolding of epithelium and marked fibroplasia.

Relationship between mortality rate and bursal lesion score

The mortality rate was detected from the farm records (Table 3). A proportional relationship between the mortality rate and the bursal lesion score was determined within the respective group.

RESULTS AND DISCUSSION

Experimental chickens/Clinical cases

The morbidity rate of the affected flocks was around 100%, and the mortality rate was variable ranging from 7-38%. Highest mortality rate was recorded in cockerel (38%) followed by layer (27%) and broiler (7 – 19%), respectively.

Grouping of the flocks

The flocks were categorized into five groups basically based on the severity of the disease and the mortality patterns. The mortality rate was determined from the farm records.

Vaccines and vaccination

The birds were vaccinated with the commercially available Gumboro vaccines containing intermediate strains of IBDV and the vaccines were administered as per instructions. The faults in the transportation, preservation, preparation and administration of vaccines and other probable managemental errors closely associated with the vaccination failure were identified and the tentative interpretation(s) of vaccination failure in connection with each suspected factor was summarized (Table 4). The level of maternally derived antibody during the administration, cold chain maintenance by the distributors, the residual pathogenicity of the vaccine virus, and failure due to variation in the antigenicity or immunogenicity, if any were not determined.

Clinical findings

The clinical signs of the affected birds of the vaccinated flocks varied from farm to farm and breed to breed. The signs were clinically characterized as anorexia, high fever, variable degrees of whitish diarrhea, depression, ruffled feathers, huddling together, and death.

Necropsy, histopathology and bursal lesion score

The gross morbid lesions of the muscles and the bursa of Fabricius as well as the histopathological lesions of the bursa of Fabricius with bursal lesion scores of the affected birds of different groups were mentioned (Table 2).

Table 2. Gross and histopathological lesions with bursal lesion scores of the different groups

Experimental group	Gross morbid lesions	Histopathological lesions	Bursal lesion score
Apparently normal	0	0	0,0,0
Broiler 1	• Swollen oedematous bursa • Absence of muscular haemorrhage	• Moderate to severe lymphoid depletion • Focal necrosis • Follicular atrophy and interfollicular oedema • Some degrees of fibroplasia	2, 4, 3, 1
Broiler 2	• Swollen oedematous bursa • Muscular haemorrhages	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular oedema with thickened serosa • Infolding of epithelium	2, 4, 4
Broiler 3	• As above	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular oedema with thickened serosa • Infolding of epithelium	4, 4, 4
Layer	• As above	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular oedema with thickened serosa • Infolding of epithelium	4, 4, 4, 4
Cockerel	• As above	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular oedema with thickened serosa • Infolding of epithelium	4, 4, 4, 4
Unvaccinated infected group	• Swollen, haemorrhagic and atrophied bursa containing caseous exudate • Severe haemorrhages in breast and thigh muscles	• Follicular atrophy • Cystic formation • Infolding of epithelium • No fibroblastic proliferation • Erythrocytes extravascularly	4, 4, 4

Table 3. Comparative study between the mortality rate and the bursal lesion score of the respective group

Experimental group	Mortality rate (%)	Average bursal lesion score
Normal	0	0
Broiler 1	7	2.50
Broiler 2	13	3.33
Broiler 3	19	4.00
Layer	27	4.00
Cockerel	38	4.00
Unvaccinated infected group	47	4.00

Relationship between mortality rate and bursal lesion score

The mortality rate and the bursal lesion scores of the different groups were different (Table 3). There was an around proportional relationship between the mortality rate and the bursal lesion score within the respective group.

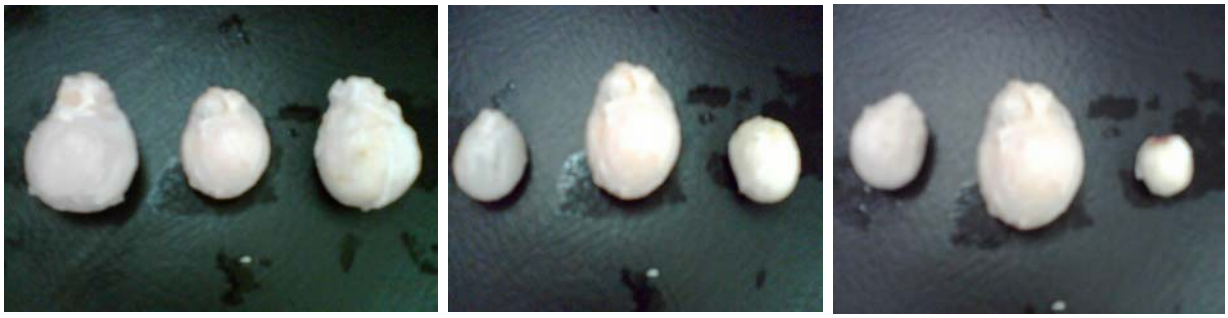
The pathogenicity of IBDV virus related to outbreaks in the vaccinated flocks and the probable causes of vaccination failure, especially in connection with defective farm managements were investigated. A number of 23 such outbreaks were recorded, the pathogenicity of the virus was determined as highly virulent strain of IBDV, and the probable causes of vaccination failure were suspected (Table 4).

Gumboro is the threat of poultry farming in Bangladesh and there is none alternative to prevent IBD without vaccination (Lukert and Saif, 1997). But Gumboro outbreaks in the vaccinated flocks were recorded elsewhere (Lukert and Saif, 1997; Hafez *et al.,* 2002). Various vaccines against IBD are commercially available. Some vaccines were tested their protection level experimentally giving challenge with vvIBDV 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 farms. Different factors related to

Gumboro vaccine failure were suspected in the present study (Table 4). However, vaccination failure in connection to variation in the antigenicity among the IBD viruses (Rosenberger *et al*., 1987), interference between the residual maternally derived antibodies and the vaccine virus (Eterradossi, 2001) and the appropriate time of vaccination were not determined in this study. 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 differ between strain to strain (Rosales *et al.,* 1989a, b, c; Abdel-Alim and Saif, 2001) and the invasive vaccine strains are able to break through higher maternally derived antibody level (Kouwenhoven and van den Bos, 1994). The genetically engineered tissue culture adapted vvIBDV was attempted to use as vaccine candidate, but the attempt was not yet successful for its reversion (Raue *et al.,* 2004).

The mortality rate determined in this study was upto 36% in the vaccinated flocks. Highest mortality recorded in layer followed by cockerel and broiler, respectively. This picture of mortality principally found in the flocks infected by vvIBDV. IBDV strains isolated from the affected chickens induce severe clinical signs with high mortality in specific pathogen free (SPF) chickens (Nunoya *et al.,* 1992; Tsukamoto *et al.,* 1992). However, the mortality rate in the unvaccinated affected flocks in this study was 47%. The clinical signs of the affected birds of the vaccinated flocks were variable (mild to the signs generally developed due to the infection with vvIBDV). The data clearly indicated the vaccination failure in the flocks. Almost a proportional relationship between the mortality rate and the bursal lesion scores of the respective group was observed in the study (Table 3). The scores of the different experimental groups clearly indicated the degree of severity of the disease which was closely associated with the pathogenicity of the IBD virus.

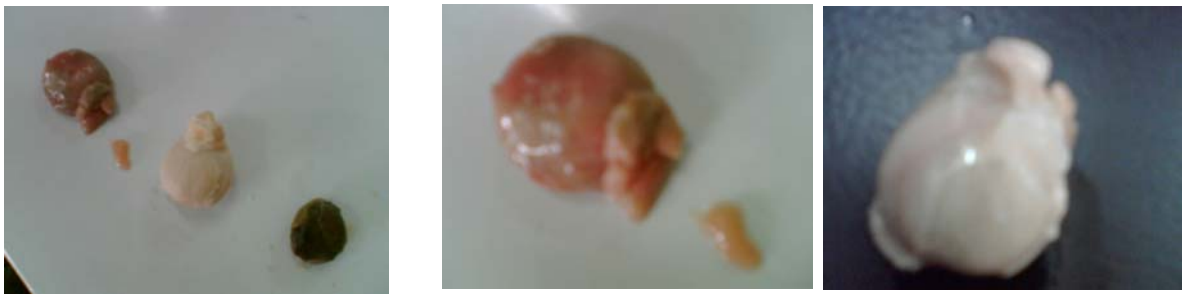
The gross and histopathological lesions of the bursa of Fabricius were variable in different experimental groups in the present study (Table 2).



Swollen: Normal: Swollen (5.7 gram : 5.5 gram : 5.8 gram)

Atrophied: Normal: Atrophied (1.2 gram : 5.5 gram : 1.3 gram)

Atrophied: Normal: Atrophied (0.7 gram : 5.5 gram : 0.4 gram)



Mild Haemorrhagic: Normal: Dark haemorrhagic (2.9 gram : 5.5 gram: 2.3 gram)

Mild Haemorrhagic bursa with exudate

Swollen oedematous bursa

Figure 1. Different pathological levels of bursa of Fabricius collected from the affected broilers with previously vaccinated

Bursal lesion scores were surprisingly high in most cases except few, where moderate bursal lesions were recorded. Depending on the residual virulence of the live attenuated viruses, some vaccine strains can cause bursal damage (Mazariegos *et al.,* 1990) and lead to immunosuppression in the vaccinated birds (Edward *et al*., 1982; Reece *et al.,* 1982). Although highest bursal lesion scores with cyst formation (Tsukamoto *et al.,* 1995), lymphocytic depletion

with inflammation (Mazariegos *et al.,* 1990), acute necrosis (Rautenschlein *et al.,* 2001), follicular atrophy (Franciosini and Coletti, 2001), extensive bursal damage with follicular repopulation (Rautenschlein *et al.,* 2001) and increased interstitial connective tissue proliferation (Franciosini and Coletti, 2001) produced by intermediate vaccine strain of IBDV were reported. The high scores of bursal lesions especially found in the outbreaks with vvIBDV (Raue *et al.,* 2004).

The histopathological features and remarkably high score of bursal lesions in this study would evaluate the virus as undoubtedly highly pathogenic virus which could either be vvIBDV or vaccine virus. However, the pathogenicity of vaccine viruses were not yet determined in a separate experiment and further experiment to evaluate it can be conducted. Several suspected factors in connection to managemental errors in this study might be closely related to vaccination failure and outbreaks in the vaccinated flocks.

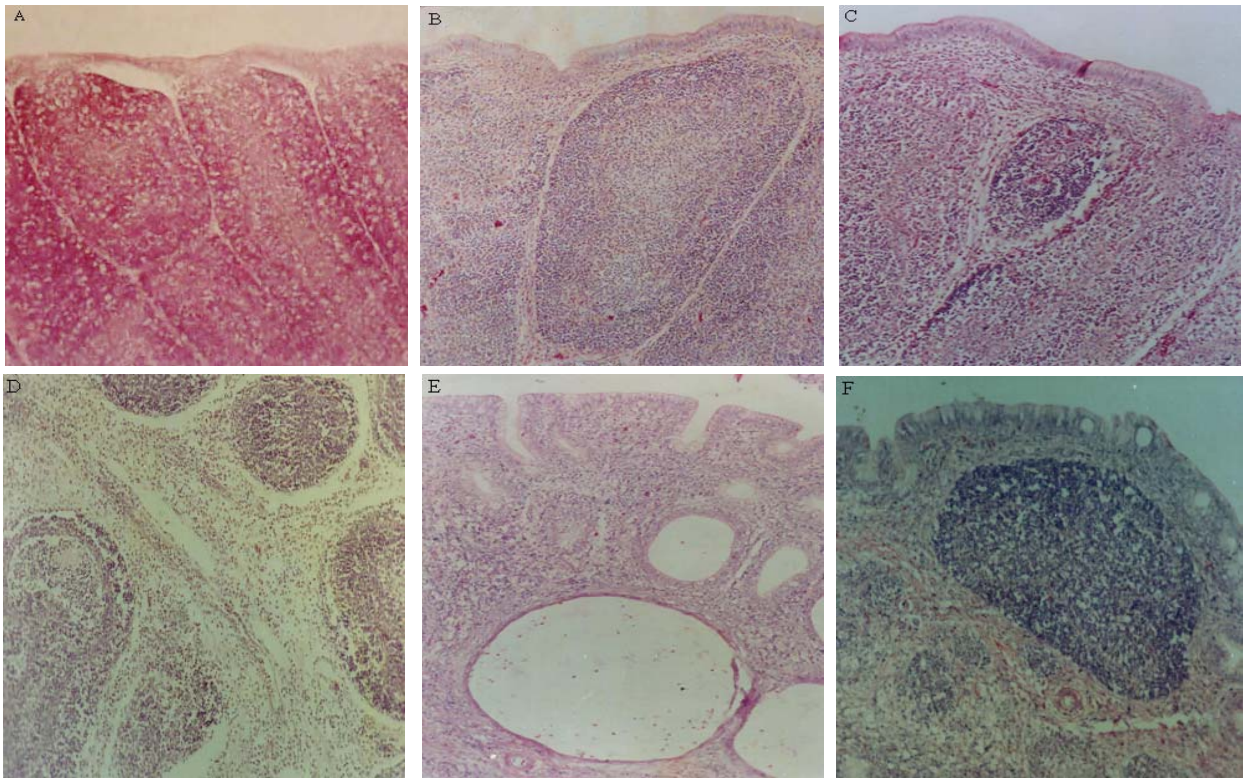


Figure 2. Histopathology of bursa of Fabricius affected by IBDV and scoring of bursal lesions; A. apparently normal lymphoid follicles (score 0), B. mild lymphoid depletion (score 1), C. moderate lymphoid depletion along with focal necrosis (score 2), D. severe lymphoid depletion with reactive cells infiltration and fibroplasia (score 3), E. atrophy of bursal follicles along with cystic development (score 4), and F. variable degree of follicular regeneration

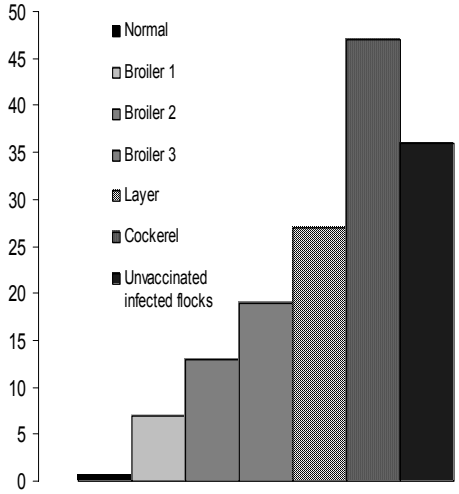


Figure 3. Mortality rate of the birds of the different groups

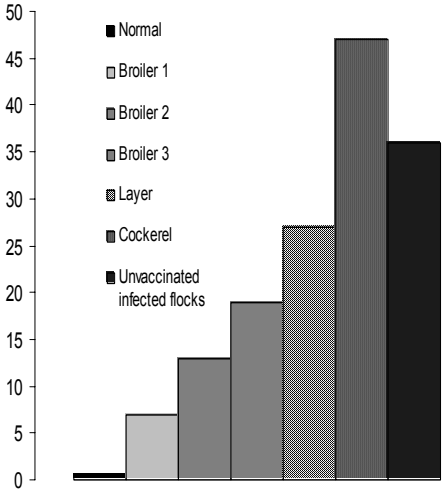


Figure 4. Bursal lesion scores of the different groups

Vaccines were most repeatedly failed in those flocks where the batches of birds reared giving at least an interval to destroy the persistent IBDV and single dosing without boosting was followed. IBDV is highly infectious and very resistant to inactivation. The viruses could survive outside the host for at least for months (Allan *et al*., 1982). Houses that contained infected birds are infective for inmate birds after 54 and 122 days (Benton *et al.,* 1967). According to Godwin (2001), the factors causing vaccine breaks are either vaccine types, storage and handling; or condition of the birds including the level of maternally derived antibodies; or administration of vaccine. In this study, vaccination failure exclusively due to defective managements were thoroughly investigated and the suspected factors were listed (Table 4).

Table 4. Suspected factors of vaccination failure with their tentative interpretations

Suspected factors causing vaccination failure	Tentative interpretations on vaccination failure	No. of incidences
Previous history of Gumboro outbreaks	Virus loads in the farms and the birds of the newly batch(es) became exposed	3
Vaccination at early age (Between 7 to 10 days of age limit)	Inactivation of vaccine viruses by maternally derived antibodies	2
Vaccination beyond the optimal age limit	Vaccination after exposure	1
Intervals between the succeeding batches not more than 10-20 days	The shaded virus could be viable and the birds of the succeeding batch(es) might be infected	3
Single dosing without boosting	Insufficient immune response	4
Cold chain break during transportation, preservation and processing	Inactivation of the vaccine viruses	1
Completion of dosing taking prolonged time	The vaccine viruses might be inactivated and subsequent infection	1
Lower dosing	Insufficient immune responses	1
Rearing of one more batches of different ages in the same farms	Continuous exposure by different sources of IBDV infection	2
Vaccination and disinfection simultaneously as spray or in drinking water	Inactivation of the vaccine virus	2
Vaccination at stressful condition	Inadequate immune response	1
Vaccination through inappropriate drinking water	Inactivation of the vaccine virus	2

However, the inactivation of vaccine virus may be due to careless transportation, preservation, preparation and administration of vaccines, and the vaccination and disinfection simultaneously by the farmers were noticed. All of these clues might be strongly associated with the vaccination failure, although the exact causes of vaccine breaks in

connection with the antigenicity, immunogenicity and pathogenicity of vaccine viruses to protect the birds from this devastating malady are still obscure.

CONCLUSION

The pathogenicity of the infectious bursal disease virus related to the outbreaks in the vaccinated flocks was observed as highly pathogenic IBDV. Mostly lacking of farmer's awareness about the vaccines and vaccination might be closely related in the vaccination failure.

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