

Immunological tolerance of Bhutanese native chicken to Infectious Bursal Disease Virus infection

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Abstract

Infectious bursal disease is a disease of economic importance worldwide. Vaccination is the only the effective method to control the disease. The selection and breeding for disease resistance is an emerging concept towards a sustainable alternative for control the disease in future. Individual chicken types/breeds are claimed to be relatively more resistant to diseases than others. The disease resistance trait and genetic resources are crucial for the development of IBD resistant breeds. The outbreak of IBD is a threat to the emerging commercial layer industry in Bhutan. However, no outbreak of IBD is reported in Bhutanese native chickens till date

. Therefore, this study assessed the tolerance of Bhutanese native chicken to IBD infection through analysis of mortality rates and serum antibody titres of chicken from IBD outbreak areas in Tsirang district, Bhutan. The average mortality rates was 35.4 percent (commercial strains) and zero (native types) respectively in native chickens in IBD affected farms. All four study groups tested positive to IBD virus with 94.7, 95.2, 80.0 and 63.6 percent for CA, CNA, NA and NNA groups respectively. The number of sero-prevalence of NNA was significantly ($p < 0.05$) lower than CA and CNA groups. The log₁₀ titres indicating protective antibody levels were not significantly different among the groups. Overall, the absence of clinical signs and no mortalities in native chicken,

sero-positivity to IBD virus infection and similar levels of protective antibody titres of native chicken groups to commercial chicken line is suggestive of potential immunological tolerance of Bhutanese native chicken to IBD virus infection.

Keywords: *serum antibody titres, disease resistance, mortality*

1. Introduction

Infectious bursal disease (IBD) is a highly contagious disease and economically an important disease of the poultry industry worldwide. The mortality rates due to IBD outbreaks ranges up to 60 percent in layers (van den Berg et al 2000) depending on virulence. Despite vaccination as an important strategy to disease management, quite often IBD outbreaks occurred in vaccinated flocks due to failure of vaccine to induce protective immunity.

The outbreak of IBD is a growing concern to the developing commercial poultry industry in Bhutan. The growing poultry industry in the country is characterized by small scale commercial farms, maintaining small flock of native chicken along with commercial strains. Native chickens of Bhutan are classified into more than 10 types (DADIS 2015) while Hyline brown strain is used as commercial layer strain. The native chicken types are mainly maintained under

scavenging system for household consumption. They are considered resistant to range of diseases although literatures on their resistance birds are scarce. Resistance to IBD infection in form of lower mortality rates vary with chicken types (Hassan et al 2004). Native chicken types in particular under experimental infection showed low mortality compared to other types (Okoye et al 1999). Further, their resistance to other poultry diseases such as fowl typhoid (Mdegel et al 1998) and avian leucosis complex (Oluyemi et al 1979) are available. Variation in disease resistance among individuals and breeds are based on immunological system and its interaction with physiological and environmental factors (Zekarias et al 2002).

Unpublished data showed that occurrence of several IBD outbreaks in mid 1990s and later in 2010 in semi commercial broiler farm Samtse, Bhutan (Zyl 2010). The recent sporadic outbreaks occurred in small scale commercial layer farms in Sarpang, Tsirang and Punakha districts in 2014. However, outbreaks of IBD in native chicken have not been reported till date in Bhutan.

The genetics and breeding or development of a strain or variety resistant to a disease requires understanding candidate resistant genes. The advantage of genetic resistance as sustainable approach to control of infectious diseases in poultry is emphasized through evolution of virulent infectious pathogens and the limitations on the use of chemotherapeutics. Therefore this study assessed the immunological tolerance of Bhutanese native chickens considering mortality and level of serum antibody titres to natural IBD virus infections in comparison to the commercial vaccinated chickens.

2. Materials and Methods

The study involved three small scale commercial layer farms with infectious bursal disease outbreak and also rearing native chicken in backyard, one small scale commercial farm with no IBD outbreak and one native chicken backyard farm with no IBD outbreak in Tsirang district, Bhutan. The IBD outbreak was reported in the district in 2014. As per the case report, the native chicken in these affected farms were reported asymptomatic of IBD during the outbreak. Therefore, a detailed information on flock size, age of outbreak, vaccination status and mortality rates in each farms were recorded. Further, a four study groups representing commercial (CA) and native in affected farm (NA), unaffected farm of exclusive commercial

(CNA) and one unaffected farm of exclusive native chicken (NNA) in the vicinity of outbreaks where chosen for sero-sampling (Figure 1) to study the serum antibody profiles. Twenty random blood samples from each group of 2ml of blood was withdrawn from the wing vein and transferred into vacutainer tubes without additives for serum.

The sera samples were used to determine IBD antibody titre using commercially available enzyme linked immunosorbent assay (ELISA) kit (IDEXX IBD KIT® (IDEXX Laboratories, Inc., 2014, USA) as per manufacturer's instruction. Briefly, the antigen-coated plates and the ELISA kit reagents were adjusted to room temperature prior to the test. The test sample was diluted 1:500 with sample diluent prior to the assay. One hundred µl of diluted sample was then loaded into each well of the plate. This was followed by 100 µl of undiluted negative control into well A1 and A2, 100 µl of undiluted positive control into well A3 and A4. The plate was incubated for 30 minute at room temperature. Each well was then washed manually with approximately 350 µl of distilled water 3 times. Goat anti-chicken conjugate (100 µl) was dispensed into each well. The plate was incubated at room temperature for 30 min, followed by washing each well with 350 µl of distilled water 3 times. Tetramethylbenzidine (TMB) solution (100 µl) was dispensed into each well. The plate was then incubated at room temperature for 15 min. Finally, 100 µl of stop solution was dispensed into each well to stop the reaction. The absorbance (A) values were measured and recorded at 650 nm. The presence or absence of antibody to IBDV was determined by relating the A 650 nm value of the unknown to the positive control mean. The relative level of antibody in the unknown was determined by calculating the sample to positive (S/P) ratio. As indicated S/P ratio (sample to positive ratio) of 0.20 or less was considered as negative while more than 0.20 was positive. A log₁₀ titre value of 3.4 was considered as protective antibody titre to challenge by virulent wild strain of IBDV as in Moraes et al 2005.

The data was entered to MS excel spread sheet program to create data base and was filtered before analysis in GraphPad Prism version 5.00 (California, USA). Descriptive statistics, Kruskal Wallis test with Dunn's multiple comparison tests was used to analyze the prevalence of disease and one way analysis of variance of log₁₀ titres. A p value < 0.05 was considered a significantly different.

3. Results and Discussion

The outbreak of IBD in the study sites occurred in commercial layer farms during May-July, 2014. The birds were 4-5 weeks of age during the outbreak. All four affected commercial farms also maintained native chickens ranging from 10-35 birds. The average mortality rate in commercial chicken type was 35.4 percent (range 24 to 50 percent) while no mortality was observed in native chicken (Table 1). Further, no clinical signs were observed in these native chickens.

Table 1: Flock size, mortality and occurrence of IBD outbreaks in Tsirang district

Farm No	Village	Chicken Type	Flock size	No. dead (Mortality %)	Outbreak (age of chicken)
Farm 1	Chuzomsa, Patshaling	Commercial	375	150(40.0)	June, 2014 (4-5 weeks)
Farm 2	Chuzomsa, Patshaling	Native Commercial	10 300	0 83(27.7)	June, 2014 (4-5 weeks)
Farm 3	Drupchugang, Tsholingkhar	Native Commercial	15 500	0 120(24.0)	June, 2014 (4-5 weeks)
Farm 4	Zomlingthang, Gosering	Native Commercial	35 300	0 150 (50)	May, 2014 (4-5 weeks)
		Native	10	0	

The mortality in CA group indicate that protective immunity was not attained through vaccination. Reinfection of IBD virus in vaccinated flocks occurs (Islam and Samad 2003; Hassan et al 2002) as a result of vaccination failures (Jindal et al 2004). However, mortality rates due to reinfection in the current study for vaccinated flock are very high compared to Hassan et al 2002 and within van den Berg et al 2000.

The lack of mortality or clinical signs in Bhutanese native chicken co-existing with CA group and also in NNA is suggestive of sub-clinical infection in native bird group. Subclinical IBD infections is reported in broiler chicken (Homer et al 1992), unvaccinated native chicken (Sawi et al 2011; Okwar, 2011; Mushi et al 2006). However, the primary infection are also unapparent when virus is of low virulence or due to presence of maternal antibodies (van den Berg et al 2000). On the other hand, CNA group is either not exposed to the virus or has remained protected through

vaccination as indicated by lack of clinical signs and mortality.

All four study groups showed positive antibody titres to IBD virus. The sero-prevalence of cases among the group was significantly different ($p < 0.05$) with NNA group significantly low compared to CA and CNA groups. The mean \log_{10} titres of CA group was the highest followed by CNA, NNA and NA groups but not significantly different ($p > 0.05$) from each other (Table 2).

Table 2: Antibody titre and mean \log_{10} antibody titre (SD)

Group	IBDV infection	(%)	Mean IBDV antibody titre (SD)	\log_{10}
CA	+ve	18 ^a	94.7	3.77 (0.179)
	-ve	1		
CAN	+ve	20 ^a	95.2	3.73 (0.137)
	-ve	1		
NA	+ve	12 ^{ab}	80.0	3.57 (0.352)
	-ve	3		
NNA	+ve	14 ^b	63.6	3.66 (0.152)
	-ve	8		

^{a,b,c} No. of positive cases as indicated by different superscripts are statistically significant difference at $p < 0.05$.

The incidence of sero-positive antibody titres and protective antibody titres in native chickens (NA and NNA) groups similar to commercial chicken groups (CA and CNA) in this study suggests exposure of native birds alike to the wild virus strain of IBD virus. A similar levels of protective antibody titres in CA group compared to CNA group and incidence of mortality in former suggests the failure to attain adequate protective antibody titres through vaccination. The sero-prevalence of IBD virus specific antibody in native groups (NA and NNA) although without clinical signs or mortality shows that the chickens were exposed the wild strain of virus as these native chicken are not vaccinated.

4. Conclusions

The lack of clinical signs and mortality, evidence of exposure to wild strain IBD virus (sero-positivity) and presence of protective antibody titres in unvaccinated native chicken similar to the affected commercial chicken type in this study suggests Bhutanese native chickens are relatively immunotolerant to natural IBD infections compared to commercial layer strains in the

country. Further study preferably experimental infection is recommended to validate the current findings.

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