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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. The vaccination is an important management tool to control the disease. However, outbreaks do occur in vaccinated flocks due to vaccination failure. The selection and breeding for disease resistance may be a sustainable approach to control the disease in future. The outbreak of IBD is a threat to the emerging poultry industry in the country. Such outbreaks have not reported in native chickens so far. Therefore, this study aimed to assess the immunological tolerance of Bhutanese native chicken from a recent IBD outbreak areas in Tsirang district, Bhutan. The mortality rates in commercial and native birds maintained under same farms were assessed. Further, the level of antibody titres to IBDV of an exclusive commercial and native chicken farm from the vicinity of outbreak using commercial kit (this statement appears incomplete). Overall, the study groups consisted of commercial affected (CA), native chicken in co-existing in commercial affected (NA), Commercial Not Affected farm (CNA) and Native not affected farm (NNA). The mortality rates commercial chicken ranged from 24 to 50 percent while no mortalities were observed in native chickens in IBD affected farms. All of the four groups were sero-positive to IBD virus although prevalence was significantly ($p<0.05$) lower in NNA group compared to CA and CNA groups. The $\log_{10}$ titres used to determine protective antibody titre levels showed no significant differences among the groups. Overall, the absence of clinical signs and mortality, sero-positiveness to IBD virus infection and levels of protective antibody titres in unvaccinated NA and NNA groups suggests of potential immunological tolerance of Bhutanese native chicken to IBD virus infection. A further study is recommended to validate the current findings possibly through experimental infection.

Keywords: *native chicken, tolerance, mortality, serum antibody titres, Bhutan*

1. Introduction

Infectious bursal disease (IBD) is a highly contagious disease and economically important disease of the poultry industry worldwide. The mortality rates depending on virulence ranges up to 60 percent in layers (van den Berg et al 2000). Despite vaccination as an important strategy to disease management, quite often IBD outbreaks occurred in vaccinated flocks due to vaccine not being able to induce protective immunity.

The outbreak of IBD is also a 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. There are about 10 strains of chicken including *Yupja Naap, Seim, Pulom*, *Shekini*, *Bailetey* (DADIS 2015). They are maintained under scavenging system for household consumption. Generally, they are believed to be resistant to range of diseases although literatures on their resistance birds are scarce.

Unpublished data showed that in Bhutan there were several IBD outbreaks in mid 1990s and later in 2010 in a semi commercial broiler farm, Samtse, Bhutan (www.poultry.net). The recent outbreak was reported in 2014 in commercial strains of bird. Outbreaks of IBD in native chicken have not been reported till date in Bhutan. Differences in response to IBD infection in different chicken types in the form of variation in mortality rates (Hassan et al 2004) and low mortality in native chicken types in particular under experimental infection (Okoye et al 1999) are reported. Further resistance of native chicken types to other diseases such as fowl typhoid (Mdegel et al 1998) and avian leucosis complex (Oluyemi et al 1979) are available. Immunological system and its interaction with physiological and environmental factors contributes to the variation in disease resistance among individuals and breeds (Zekarias et al 2002). Some of the antiviral genes are upregulated in response to IBD infection and are associated to resistance (Smith et al 2015).

Disease resistance/tolerance is an important trait considered for a sustainable control of infectious diseases in poultry. Further, evolution of virulent infectious pathogens and the limitations on the use of chemotherapeutics, emphasize the advantage of breeding for genetic resistance. Therefore this study was aimed to study immunological tolerance of Bhutanese native chickens through assessment of the mortality and level of antibodies titres in the serum to natural IBD virus infections relative to commercial vaccinated chickens.

2. Materials and Method

Outbreak of IBD in four small scale commercial layer farms under Tsirang district, Bhutan was reported in 2014. The farms maintained flock of native chicken for household consumption. The native chicken in these farms were reported asymptomatic of IBD. Therefore, detailed information on farm management, date and age of outbreak and mortality were recorded in four affected farms. In addition to these farms representing affected farm for commercial (CA) and native (NA) groups, one unaffected farms of exclusive commercial (CNA) and one unaffected farm of exclusively native chicken (NNA) groups in the vicinity where chosen for sero-sampling (Figure 1). Twenty random samples from each group consisting of 2 ml of blood was withdrawn from the wing vein and transferred into vacutainer tubes without additives for serum.

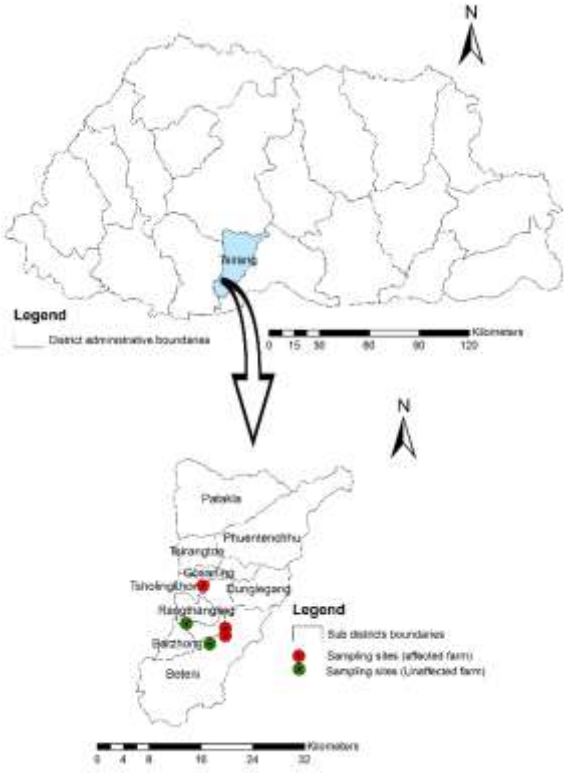

Figure 1. Infectious Bursal Disease Outbreak and Sampling sites

The sera samples were used to determine the 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. (Please insert the ELISA methodology here) 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 (Moraes et al, 2005) was considered as protective antibody titre to challenge by virulent wild strain of IBDV. The incidences of positive and negative were analyzed using Kruskal Wallis test with Dunn's multiple comparison tests and one way ANOVA for log10 titre was performed using GraphPad Prism version 5.00 (California, USA). A P value <0.05 was considered a significantly different.

3. Results and Discussion

The outbreak developed in commercial strains at the age of 25 days old in affected farms and during May-July, 2014. All four affected farms composed of both commercial and native chickens. The mortality ranging from 24 to 50 percent were observed in commercial chicken. The number of native chicken per farm ranged from 10-35 (Table 1). There were no clinical signs or mortality observed in native chickens of similar age maintained in the same farm.

Table 1. Flock size and mortality during IBD outbreaks in Tsirang district, Bhutan

Farm	Village	Chicken Type	Flock size	No. dead (Mortality %)
Farm 1	Chuzomsa,	Commercial	375	150 (40.0)
	Patshaling	Native	10	Nil
Farm 2	Chuzomsa,	Commercial	300	83 (27.7)
	Patshaling	Native	15	Nil
Farm 3	Drupchugang,	Commercial	500	120 (24.0)
	Tsholingkhar	Native	35	Nil
Farm 4	Zomlingthang,	Commercial	300	150 (50)
	Goserling	Native	10	Nil

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 range of 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 chicken 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 indicate 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 $\log_{10}$ IBDV antibody titre (SD)
CA	19	+	18[a]	3.77 (0.179)
		-	1	
CNA	20	+	20[a]	3.73 (0.137)
		-	1	
NA	15	+	12[ab]	3.57 (0.352)
		-	3	
NNA	22	+	14[b]	3.66 (0.152)
		-	8	

[a,b,c] Number of positive cases as indicated by different superscripts are significantly different 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 chicken 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 IBDV (sero-positivity) and protective antibody titres similar to the affected commercial chicken type in farms with IBD outbreak in this study suggests Bhutanese native chickens are relatively immunetolerant 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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