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# Seroprevalence of Infectious Bursal Disease in Non-vaccinated Village Chicken in Jigjiga and Harar Districts, Eastern Ethiopia

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## Abstract

Cross-sectional study was carried out from October 2014 to April 2015 to determine the sero-prevalence of infectious bursal disease (IBD) in non-vaccinated village chickens of Jigjiga and Harrar Districts, Eastern Ethiopia. Serum sample was collected from 431 village chickens raised in a traditional management system in the study area. In the present study Indirect enzyme-linked Immunosorbent assays was employed to determine the seroprevalence of IBD. In the current study an overall seroprevalence of 51.7% (223/431) with an optical density (OD) reading between 0.29-0.780 nm was recorded. The study also revealed that seropositivity to IBD virus was significantly influenced by location ($\chi^2$=23.791, P<0.05). But no significant difference was observed between age groups ($\chi^2$=13.959, P=0.999), sex ($\chi^2$=17.793, P=0.153) and breed ($\chi^2$=15.004, P=0.553) on the prevalence of IBD. This study concluded that IBD is widely distributed in the current study area, thus detailed surveillance should be carried out in order to put in place appropriate control and prevention strategies.

**Keywords:** Chickens; ELISA; Infectious bursal disease; Seroprevalence

## Introduction

Infectious bursal disease (IBD) is caused by IBD virus (IBDv) which especially elicits a highly contagious infection of young chickens [1]. Poultry production systems of tropical regions are mainly based on the scavenging indigenous chickens found in virtually all villages and households in rural area [2]. The first outbreak and existence of IBD in Ethiopia was reported in Debrezeit in poultry farms in broiler and layer chickens with seroprevalence 93.30% [3]. A case-report study from Andasa poultry indicated 100% seroprevalence and overall mortality of 72% in young (1-70 days old) and 7% in adult (>70 days old) in non-vaccinated flocks [4]. Zegeye et al. [5] reported a 45.05% overall seroprevalence of IBD in backyard chickens in Mekelle, Northern Ethiopia. Tesfaheywet and Getnet et al. [6] reported seroprevalence of 82.5% in Debrezeit, central Ethiopia. Furthermore, Kassa and Molla et al. [7] have also reported seroprevalence of 73.5% in northern Gonder. Even though indigenous chickens are known to possess desirable characters such as thermo tolerant, resistant to some disease, good egg and meat flavor, hard egg shells, high fertility and hatchability as well as high dressing percentage, their productivity is still very low due to various risk factors including diseases [8]. Among the different viral diseases of chickens, infectious bursal disease (IBD), Newcastle diseases, Marek's disease, avian influenza, fowl pox and infectious bronchitis are common. Infectious bursal disease is among these viral diseases that causes damages in the poultry production [9]. The IBD virus primarily targets lymphoid tissue and results in extreme kidney damage in birds that are infected [10]. Although the disease is a major health constraint responsible for marked economic losses in a country, its status in chickens in the study areas have not been yet studied to full extent. Therefore, the objective of this study was to determine the seroprevalence of infectious bursal disease in selected areas of Eastern Ethiopia.

## Materials and Methods

### Description of the study areas

The study was conducted in two selected town of Eastern part of Ethiopia namely: Jigjiga, and Harar from October 2013-April 2015. Jigjiga is located 615 km far from Addis Ababa in East direction at an altitude of 1100 m above sea level. The area is located at 9° 21′N 42° 48′E. It is characterized by mild subtropical weather, average minimum and maximum temperatures of 28 and 34°C respectively. This area experiences a binominal rainfall pattern with a short rainy season from June-September and long dry season from November-April. On the other hand, Harar is located 526 km far from Addis Ababa in East direction at a latitude of 8° 50′, 9° 15' N and longitude of 9° 36′N 41° 52′E at altitude of 1850 m above sea level. The annual rainfall of the area is between 834-1300 mm and annual temperature of minimum and maximum, 21 and 26°C, respectively. Rainy season occurs with bimodal distribution of which 70% occurs during the main rainy season (June-September) and 30% during the small rainy season (March-April). The relative humidity of Harar is 50.4% [11].

### Study design and study animals

A cross-sectional study was conducted in reared local backyard poultry production and management system with taking into consideration of their age, sex, breed and location. The chickens were categorized into local and exotic breeds and chickens of both sexes were included in the study. The ages of the chicken were classified as adults (>12 months) and young (6-12 months). In this study chick less than 6 months were not found in the study area. The ages were determined subjectively based on the size of crown, length of spur and

flexibility of the xiphoid cartilage according to Magwisha et al. [12] together with information from the poultry farmers.

### Sample size determination and sampling method

Since there was no prior similar research work conducted in the study area, expected prevalence was assumed to get the maximum number of sample size required. The absolute precisions were decided to be 5% at 95% confidence level. Thus, for sample size estimation, the formula described by Thrusfield et al. [13,14] was used. Accordingly, a sample size of 384 was obtained using formula. However, the sample size was increased by a factor (0.12) to increase the precision of study as well as to compensate for loss of blood samples thereby making the total number of chickens to be 431.

Selection of sample was made using a deliberate unbiased process. So, multistage cluster sampling procedure was followed to get sampled birds. This was conducted by dividing the study population into exclusive groups and then number of sampling units selected from each stratum. Study sites were selected based on the existing epidemiological situations and following the route of poultry dissemination from multiplication centers. Accordingly, the 431 chickens were systematically selected from 7,358 chickens in the backyard production system of the selected Pas/kebeles. Systematic sampling methods were applied after sampling interval was determined using the formula K=N/n. Where: N=represents estimated total chickens for backyard farm in sampling frames; n=allocated sample size and K=interval of household to be sampled (Pfeiffer, 2002). Accordingly, at every 17 household intervals a chick was caught and examined.

### Sample collection

A total of 431 serum samples were collected from village chicken in the study area. All the serum samples were heat inactivated at 56°C for 25-30 minutes in a water bath and then processed by ELISA. Out of these 431 samples, 170 were from Jigjiga and 261 from Harar village chicken. Three ml of blood were collected from the jugular vein using sterile 5 ml capacities of disposable syringe and with needle size 22 (gauge) × 1¼ following the method described by Alcorn [15].

### Laboratory diagnostic methods

**ELISA test procedure, validity and interpretation:** Enzyme-linked Immunosorbent assays (ELISA) was performed at the National Veterinary Institute (NVI), Deber-zeit, Ethiopia, using a commercial available blocking ELISA kit (Proflock plus infectious bursal disease virus (IBDV) antibody test kit) to detect specific antibodies against IBDV according to the manufacturer's manual. Briefly, both the sera samples that were preserved at -200°C, the antigen reagents that was preserved at 40°C were adjusted to room temperature of 22-27°C prior to the test. All the serum samples were heat inactivated at 560°C for 30 minutes in a water bath (Rahman et al., 2004). Sera samples were diluted by adding 500 µl of the sample diluents to each 1 µL of the serum sample prior to the assay using pipette with disposable tips. 100 µl of diluted sample was added into each wells and 100 µL of undiluted negative control into well A -1 and well A -2, and 100 µL of undiluted positive control into well B-1 and well B-2, the plate was then covered with lid and incubated at room temperature of 220°C for 30 minutes later on the contents of wells were aspirated and each well was washed with 300 µl of wash buffer for 4 times and wells in which 100 µl of the conjugate reagent was added into each well and the plate was covered with lid and incubated at room temperature of 220°C for 30 minutes, the contents of the wells were then aspirated and washed 4 times and the plate was inverted and taped firmly on absorbent cloth to dry. Another 100 µl of the substrate reagent was added into each well and the plate was covered with lid and incubated at room temperature of 220°C for 15 minutes, after which 100 µl amine buffers was added into each well. The absorbance values were measured and recorded at wavelength 450 nm using spectrophotometer. The IBD antibodies titer and sample absorbance to sample to positive ratio were calculated to interpret the results according to the manufacturer's instructions.

**Validity:** IBD ELISA results are obtained when the average optical density (OD) value of the normal control serum is less than 0.250 and the corrected positive control value range is between 0.250 and 0.900. If either of these values is out of range, the IBD test result should be considered invalid and the samples should be retested. Optical density value range of normal control serum was between 0.07-0.2 and for positive control serum 0.306-0.82. For interpretation of the test results, a sample to positive ratio (s/p) of each test serum was required. Then the sample to positive ratio was calculated by the following formula directed by the manufacturer.

SP=Sample absorbance-average normal control absorbance/ corrected positive control absorbance.

The IBD ELISA titer was calculated by the following suggested equation

Log10 Titer=(1.172 × Log10 Sp)+3.614

Then, Titer=antilog of log10 titer.

### Data management and analysis

Laboratory results were entered and managed using Microsoft Excel 2010, Duxbury Press. Seroprevalence was determined using the total number of positive sera samples divided by the total number of sera tested. For analysis of serological data, the chickens were divided into 2 groups: those with ELISA OD value less than <0.3 and those with greater than or equal to 0.3 (protective against IBDV). Statistics were employed using statistical software program namely statistical package for social science (SPSS) version 18.0 [16,17]. The Chi-square test was used for the association of OD and risk factors.

## Results and Discussion

### Overall seroprevalence of IBD

Optical density readings of serum samples collected from animals was ranged from 0.01-0.760 nm while, OD of the negative and positive control sera were 0.0845 nm and 0.300 nm respectively. In the present study, of the total serum samples examined, an overall seroprevalence of 51.7% was recorded.

### Seroprevalence of IBD with respect to age, sex, breed and area

**Area wise seroprevalence:** seroprevalence of IBD in chickens in Harar and Jigjiga were 40% (100/250) and 68% (123/181) respectively, and the difference was statistically significant (P<0.05).

**Sex wise prevalence:** As indicated in the Table 1 below, the seroprevalence of IBD in chickens in the male and female chickens was

49% and 54.3%, respectively; however, the difference was not statistically significant (P>0.05).

**Breed wise seroprevalence:** In the present study, the seroprevalence of IBD in the local and exotic breed was 52% and 50.8%, respectively, nonetheless, the difference was not statistically significant (p>0.05).

**Age wise seroprevalence:** In the current study the seroprevalence of IBD was 33.8% in young chickens (<12 months) and 78% in older chickens (>12 months), but the difference was not statistically significant (p>0.05).

| Risk Factors | | No. of Samples examined | Sample to positive Ratio | | χ2 -test | P-Value |
|---|---|---|---|---|---|---|
| | | | (S/P ratio 0.3) | | | |
| | | | n | % | | |
| Sexes | female | 219 | 119 | 54.3 | 17.793 | 0.153 |
| | Male | 212 | 104 | 49 | | |
| Age (months) | 6-12 | 257 | 87 | 33.8 | 13.959 | 0.999 |
| | >12 | 174 | 136 | 78 | | |
| Breed | local | 260 | 136 | 52.3 | 15.004 | 0.553 |
| | Exotic | 171 | 87 | 50.8 | | |
| Areas | Harar | 250 | 100 | 40 | 23.791 | 0.018 |
| | Jigjiga | 181 | 123 | 68 | | |
| Total | | 431 | 223 | 51.7 | | |

**Table 1:** Sera samples positive for IBD antibody in unvaccinated chickens.

## Discussion

A number of sero-diagnostic tests are available for the detection of the serum antibodies against IBD [18]. Enzyme linked immune sorbent assay has been reported to be very sensitive in the diagnosis of IBD in chickens [19]. The present study revealed that of the 431 chicken sera samples, 51.7% (223/431) samples were positive for IBD antibody. The presence of IBD antibody in these chickens might be as a result of survival from natural infection. However, maternal antibodies to IBD in unvaccinated chickens persist in chicks up to 21 days as determined by ELISA with complete decay by 28 and 35 days [20]. Antibody detected in these chickens cannot be maternally derived because the age range of birds used for this study was conducted on age groups above 6 months. With maternally derived antibody and vaccination ruled out, the antibody detected in the chickens would have been caused by a field virus, since the chickens were on free range. This implies that the field virus is capable of inducing a higher antibody titer level. This could have occurred in age group of 20-24 weeks than less age group because of frequent exposure of the chicken and constant re-infection with the field virus in the environment. This finding agreed with reports from other areas of the country with similar back yard chicken production systems [21-23]. The result of this finding (51.7%) was in close agreement with various serological studies conducted by different researchers in different parts of the country like Mazengia [24] from Bahir Dar and Farta districts (51.10%) and Tesfaheywet and Getnet [6] from Addis Ababa Kalliti (53.3%). Similarly, the seroprevalence obtained in the current study was comparable to the reported prevalence of 50% in village chickens in Sahel zone of Nigeria [25]. Other similar reports includes that of Swai et al. [26] in Tanzania (54.8%) and Ndanyi et al. in Kenya [27] (49.3%) by using AGID as diagnostic tool.

The seroprevalence of the present study finding is slightly higher than what was reported by Tsai and Lu et al. [28] in Taiwan (45%), Singh and Dhawedkar et al. [29] in India (46.2%). In Ethiopia far higher seroprevalence was reported by Tesfaheywet and Getnet [6] in Deber-zeit (82.5%) and Kassaa and Molla [7] in north Gonder (73.5%) using indirect ELISA and Agar gel immuno-diffusion test respectively. In the contrary, the present study finding was lower compared with Ibrahim and Tanya [25] from Nigeria (60.6) and Kelly et al. [22] from Zimbabwe (55%). On the other hand, the overall seroprevalence of IBD in the present study was far higher than the result reported by Reta [30] in East Shoa Zone (39.2%) using Agar gel immuno-diffusion test, Mahasin and Rahaman et al. [31] in Sudan (30.7%) and Mushi et al. [32] in Botswana (30%). Lower prevalence rates were also reported in indigenous village chickens in Cameroon (33.9%) [33], in backyard chickens in Zimbabwe (55%) [22], and in Pakistan (34%) [34]. Overall, the discrepancies between the findings of the present and the previous studies could be attributed to the difference in the test employed, serological survey results can vary depending on sensitivity and specificity of the diagnostic tool applied [35] and ELISA test is known to be highly sensitive than that of AGID [36].

In this study numerically higher prevalence was recorded in male chicken (54.3%) than female ones (49%), however, the difference was not statistically significant (P>0.05). This finding was similar with that of Reta et al. [30], who reported the absence of influence of sex on the prevalence of the disease. The prevalence obtained among male and females shows that both males and females can be infected by this contagious virus which makes vaccination the only possible control measure. Seroprevalence of young (33.8%) and adult (78%) among sampled chicken were suggestive of horizontal transmission among the age groups of chicken that are reared together. The rearing of village chickens of different age group together could make the infection

within a given flock a permanent phenomenon as suggested by Nawathe and Lamorde et al. [37]. The present study also revealed no significant difference ($p>0.05$) between the seroprevalence of IBD in local and exotic breeds of chickens. This might be due to the reason that chicken are allowed to scavenge in similar environment irrespective of breed, sex and age in the backyard production system.

The seroprevalence in the present study areas were found to be 40% and 68% for Harar and Jigjiga respectively and the difference was statistically significant ($P<0.05$). The relatively higher seroprevalence in Jigiga was indicative of a high virus activity. Conversely, the low seroprevalence in Harar was indicative of low virus activity. Since, infectious bursal disease can be transmitted through contact exposure [38]; it is probable that the high virus activity was due to horizontal transmission [39] that occurred around much waste produced by the densely populated settlements of Harar. The relatively higher IBD virus antibodies in Jigjiga chickens may be attributed to a number of factors like: the management system in traditional poultry production might support prevalent infection, poor sanitary conditions, continuous exposure of chickens to range conditions and wild birds, nutritional deficiencies and contact of village chickens with those in other villages may facilitate the spread of IBDV. This is in agreement with a previous report by Smith et al. [40]. The ease of contact at local open-air markets between chickens from different areas, which are then taken back to various localities, can undoubtedly facilitate the rapid spread and persistence of IBD among indigenous chickens. This is also in line with the nature of the disease, as there is no specific environmental situation that can prevent or modify the occurrence of the disease. The disease occurs worldwide in all major poultry production areas and it can be serologically evident in all age groups [41].

## Conclusion

The present study revealed a high seroprevalence of IBD in the study area, which could seriously affect the rearing of chickens in the backyard production system. The disease was found to have a higher seroprevalence in Jigjiga than Harar, which requires a serious attention. Hence, advising famers to get their chickens vaccinated is a necessary step in reducing the prevalence besides maintaining hygienic condition of environment in which the chickens are reared. Furthermore, it is important to fully characterize and identify the strains of viruses through sequencing of the circulating viruses in the areas and continuous surveillance should be implemented for better understanding of the epidemiology of the diseases.

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