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Sero-Prevalence of Infectious Bursal Disease in Backyard Chickens at Selected Woredas of Eastern Ethiopia

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Abstract
A cross-sectional study was conducted from November 2012 to May 2013 to determine the seroprevalence of Infectious Bursal Disease (IBD) in backyard chickens and assess the potential risk factors at selected Woredas of Eastern Ethiopia. The multistage sampling method involved for collection of serum samples and serological test (using ProFLOK® PLUS indirect enzyme-linked immunosorbent assay (ELISA)) kits was conducted at National Veterinary Institute. Out of 552 serum samples tested, 458 (83%) were positive for the disease. Among the assessed risk factors, age, breed and Woredas were statistically significant difference with the occurrence of the disease ($p<0.05$). However, no statistical significant difference was observed between sex group ($p>0.05$). Higher seroprevalence of the infection was recorded in 3-6 weeks, 360 (94.5%) age groups. Based on breed wise prevalence, cross breed (90.5%) was highly infected than local breed. The highest prevalence was recorded in Wenji woreda (92.1%). In general the study indicated high prevalent of Infectious Bursal Disease in the study areas. Thus, further study should be conducted to determine whether the chicken population in the study area needs vaccination or not.
Keywords: Seroprevalence, Infectious Bursal Disease, Indirect Enzyme-Linked Immunosorbent Assay Backyard Chicken, Central Ethiopia

Introduction

Ethiopia has large population of chicken, estimated to be 42 million. Recent estimates put the poultry population in Ethiopia at around 40.6 million with native chicken of none descriptive breeds representing 96.6%, hybrid chicken 0.55% and exotic breeds of chicken mainly kept in urban and peri-urban areas 2.84%. From the total population of chicken in Ethiopia, 99% are raised under the traditional backyard system of management, while 1% is under intensive management system (CSA, 2009).

Ethiopian poultry production has a long traditional practice which is mainly used as an immediate cash income for the rural communities although careless production system is practiced. Especially, women are more involved in keeping backyard chickens for egg collection and selling adult chickens so that this extensive breeding practice has a significant role in the livelihood of the farmers although managed poorly. Important factors in continued growth of the poultry industry in many countries are the efficiency of poultry in converting vegetable proteins into animal protein, the attractiveness and acceptability of poultry meat and egg to many people (Zeleke *et al.,* 2005).

The sector is growing more quickly than any of the other major agricultural sectors in Ethiopia. Therefore, this sector will be expected to satisfy the future demands for protein in the country. In spite of the existence large population of chicken and potential future expansion of the poultry industry in the country, the production system has been adversely affected by a variety of constraints such as management problem (like nutrition, housing), predators and poultry diseases. Among these, the diseases are the major factors that hinder poultry development and poultry mortalities due to disease are estimated to range from 20% to 50% but they can raise as high as 80% during epidemics (OIE, 2004; Safari *et al.,* 2004).

Infectious bursal disease (IBD) is one of the newly emerging disease threats to chicken in different corners of Ethiopia as described by (Zeleke *et al.,* 2005) that the disease has been speculated to be introduced concurrent with the increased number of commercial state and private poultry farms flourishing in the country and causing reduction of both the number and productivity in the sector (Aschalew *et al.,* 2002). It is caused by a virus of the genus Avibirnavirus of the family Birnaviriidae and characterized by its acute highly contagious viral disease of young chicken (Babiker *et al*., 2008). The virus has double stranded ribonucleic acid virus (RNA) with two segments A and B. Only serotype 1 appears to be pathogenic to chickens (Baxendale, 2002). Antigenic and pathogenic variant strains have been documented. The basis for emergency antigenic and pathogenic variant strains is genetic mutations in the genome of the virus. Because of the resistant nature of the IBDV, once a poultry house becomes contaminated, the disease tends to recur in subsequent flocks (Butcher and Richard, 2003).

IBDV has a worldwide distribution, occurring in all major poultry producing areas. During the 63rd general session of the Office International des Epizooties (OIE, Paris, 15 to 19 May, 1995), it was estimated that

IBD has considerable socio-economic importance at the international level, as the disease is present in more than 95% of the member countries (Eterradossi, 1995). The report of introduction and existence of IBD in Ethiopia has recently come with the report of IBD outbreak in Debre Zeit large scale poultry farms in 20 to 45 days old broiler and layer chickens and indicated that the mortality rate of the disease ranges from 45 to 50%; however, an overall of 49.83 and 93.30% was recorded for mortality rate and seroprevalence of IBD antibody, respectively (Zeleke et al., 2005b). The case report study at Bahir Dar and Farta areas indicated an incidence rate of 29.40 and 21.70% in backyard chickens in an outbreak in Debre Zeit (Zeleke *et al*., 2005b; Mazengia *et al.,* 2009).

Even though IBD is one of the important viral diseases prevalent in Ethiopia, there is limited well documented information on the prevalence and associated risk factors of IBD so far in the backyard chicken production system in eastern shewa. Thus, the study was designed to determine the seroprevalence of IBD and assess the potential risk factors in Central Ethiopia.

Materials and Methods

Study area

The study was conducted in Oromia regional state, East shewa zone at Adama City which is found about 99 km from Addis Ababa, the capital city of Ethiopia. The City is located at $08^0$33N $39^0$16E. Adama is located on the main roads of Addis Ababa to Dire Dewa road. In addition, the Ethio-Djbouti Rail way that crosses and the number of population the City are 300,000 (CSA, 2009). Moreover about 25,000 estimated people visit every day. The populations of the city are increasing from time to time so that the demand of eggs and meat consumption is rising from time to time. The area is conducive environment for chickens' production in which different industrial byproducts (feeds) are available. It is important commercial city which has a uni modal pattern of rainfall with the main rainy season extending from June to September and short rainy seasons from March to May with an average annual rain fall of 800 mm. The average annual temperature is 21°C (NMSA, 2013).

Study population

The study animals were apparently healthy and unvaccinated backyard chickens that were found in different of Kebelles and Peasant Associations (PAs) or Woredas of East shewa zone. The chickens were categorized into two age groups (0-3 weeks and 3-6 weeks), this category of age groups were made based on the development of the bursa of Fabricius that make difference on the susceptibility of the age groups to IBD (De Hedt *et al.,* 2005). And the study chickens were also categorized into two sex groups (male and female). The assessment of vaccination status of the selected chickens to be sampled was made by performing thorough questionnaire survey for each and every household

Study design

A cross sectional study was conducted from November 2012 up to May 2013 to determine the prevalence, economic significance of the IBD and associated risk factors in the study area.

Sampling methods and sample size determination

Multi-stage sampling technique was preferred to select the sampling units and the sample size was calculated according to Thrusfield (2005) formula. The Woredas and kebeles were considered as primary unit, the herds as secondary units and individual animals as tertiary units. Four districts were selected from East shewa zone. 76.5% expected prevalence was taken (Degefa *et al*., 2010) to determine samples size that should be selected with 95% confidence interval (CI) and 5% desired absolute precision.

$$n=\frac{1.96^2 (p) (1-p)}{d^2}$$

Where n= sample size
p= Expected prevalence
d= Desired level of precision (5%)

By considering the correction for multi-stage sampling design effect (multiply of 2), the final sample size come to [276x2] = 552 study animals were included.

Study methodology

Blood samples were collected from a total of 552 study animals in the study areas during the study period while Laboratory analysis of specimens was made in National Veterinary Institute (NVI). The blood samples were collected from the branchial (wings) vein of apparently healthy chickens aseptically. About 2-3 ml of blood was collected using 5 ml sterile disposable syringe with 22gauge and 11/4 needle size according to Alcon (2002). The blood was allowed to clot for 3-4 hrs at 4^0c then the syringe was placed horizontally at 45^0 to allow sera separation. The separated serum was transferred into each labeled sterile Cryovials tube and then kept cool for transportation to NVI, Debre zeit. The sera in the tube were centrifuged at 1000rpm for clarification and then the sera were stored at -20^0c until being tested. Then each serum samples were subjected to the laboratory test

through the OIE recommended diagnostic tool. The serum was tested at NVI.

Serological test procedures

Indirect Enzyme Linked Immunosorbent Assay (ELISA) was performed in NVI on all sera samples collected according to OIE Manual (2004). The antigen was obtained from Institute Pourquier, Montpellier, France. The test was conducted in NVI in Debre Zeit Veterinary libratory. The validity of the test was determined when the average optical density (OD) value of negative control <0.25 and the corrected positive control serum value ranges is between 0.25 and 0.9. If OD value is out this range the range is considered as invalid. The OD value of normal control serum ranges from 0.08-0.2 whereas positive control serum is 0.4-0.85. The serum positive control ratio was required for interpretation.

$$SP = \frac{\text{Sample absorbance} - \text{Average normal control absorbance}}{\text{Corrected Positive Control Absorbance}}$$

If SP (sample positive control) value was ≥0.5, the IBD antibody status was considered to be positive, but if <0.5 was taken as negative result.

Data Management and Analysis

All the data that was collected are entered to MS excel spread sheet program to create data base and it was filtered before analyzed by using SPSS version 20. Descriptive statistics was used to determine the prevalence of the disease and Chi-square test was used to determine any association between the disease with age, sex and body condition score and origin. In all the analyses, confidence level was held at 95% and P<0.05 was set for significance.

Results

Sex wise seroprevalence of IBD

A total of 552 sera were tested by I-ELISA and the overall sero-prevalence of IBD was 83% recorded in the study area. Out of sera examined, 243 (83.8%) and 215 (82.1%) were positive for IBD in male and female chicken respectively. The result revealed that the disease has no statistically significance variation between sexes (p>0.05) (Table 1).

Age wise seroprevalence of IBD

The sero- prevalence of IBD based on age wise was found to be 360 (94.5%) and 98 (5.73%) in 3-6 weeks and 0-3weeks age groups of chicken respectively. Higher seroprevalence of the infection was recorded in younger chicken age group. There was statistically significant difference with the occurrence of IBD infection (P=0.000) (Table 2).

Breed wise seroprevalence of IBD

The sero- prevalence of IBD based on breed wise was found to be 353 (81.0%) and 105 (90.5%) in local and cross breeds respectively. Higher seroprevalence of the infection was recorded in cross breed chicken. There was statistically significant difference with the occurrence of IBD infection (P=0.017) (Table 3).

Woreda and Kebeles wise seroprevalence of IBD

Woreda wise determination of the IBD was conducted and highest prevalence was recorded in Wenji (92.1%). There was also statistically significant difference with the occurrence of IBD infection (P=0.002) (Table 4).

Kebeles wise determination of the IBD was conducted and highest prevalence was recorded in Qobo Lixo (89.2%). There was also statistically significant difference with the occurrence of IBD infection (P=0.07) (Table 5).

Discussion

The present study, the overall of presence of IBDV specific anti body was found to be 83% in non vaccinated backyard chickens at selected Woredas and Kebeles of Eastern Shewa zone during the study period. The finding was slightly higher than other finding that were done by Kassa and Mola (2012) who reported, 75% in North Gondar and West Gojjam of northern Ethiopia, Zeryehun and Fekadu (2012) who reported 82% in central Oromia, Swai et al. (2011) who reported 82.5% in northern Tanzania, Degefa et al. (2012) who reported 76.6% in western shewa of Oromia regional states of Ethiopia, Mazengia (2008) who reported an overall prevalence of 51.1% from Bahir Dar and Farta districts and Reta (2008) who reported 76.3% in the Non Vaccinated backyard Chickens using I-ELISA test. However, the result was lower than the report of Zeleke et al. (2005b) and Woldemariam and Wossene (2007) indicated high seroprevalence rate of IBD that was 93.3 and 100% respectively. The might be due to the difference, management system, lack of awareness and study area. Highly sensitive and specific ELISA kit was used in the present. ELISA test is considered as an ideal serological test in the diagnostic virology all over the world due to its specificity, sensitivity, simplicity and minimum time requirement (Beared, 1989).

Some authors also reported lower seroprevalence rate of IBD from different countries. Tesfaye (2008) reported a seroprevalence rate of 29% by using agar gel immune diffusion (AGID) test as diagnostic tool. Moreover, Tsai and Lu (1993) and Singh et al. (1994) also indicated a seroprevalence rate of 45 and 46.2%, respectively. This difference might be due to less sensitivity of AGID as compared to ELISA kit used. This is in agreement with the Manual of Office International des Epizooties (OIE, 2004), that described ELISA as the most

ideal, sensitive and specific diagnostic tool used for serological diagnosis of viral antibodies. In addition, the present backyard chicken was managed under poor management system due to the owners hadn't given attention. In this study, age, breed and districts were found to be the potential risk factors and there was statistically significant difference observed ($P<0.05$). This result was in line with the previous finding of Degefa et al (2012), Jembere et al. (2012) and Zeryehun and Fekadu (2012). Higher seroprevalence was found in 3-6 weeks age group of chicken (94.5%). while the lowest was recorded in younger < 3 weeks age group (5.73%). This variation might be due to the difference in the development of bursa of Fabricius, which is mature and maximum size reached at age of 3 to 6 weeks. This because, chickens of age 3 to 6 weeks were more susceptible to IBD and the bursa is the site for IBDV multiplication, is matured and maximum in size at this age (Saif et al., 2000).
In the present study, the highest seroprevalence of IBD was found in Wenji woreda (92.1%). This might be due to the poor management of the backyard chickens and high contact to the stressful external environment compared to the other districts recorded having low seroprevalence in Adama (75.3%) and Modjo (76.6%) respectively. However, there was no statistically significant difference recorded between sexes ($P >0.05$) and the rate of infection was slightly higher in males (83.8%) than in females (82.1%) in this study. This might be due to the fact that both sexes have equal probability of exposure to IBD infection. This finding was in line with the previous studies (Degefa et al., 2012; Jenbere et al., 2012).

Conclusion

The present study indicated that IBD is prevalent among non vaccinated backyard chicks in the study area. This result was indicated that the chicken populations in the study area have been exposed to the virus sometime in the past, because the virus is constantly circulating in the environment. However, the level of antibodies is not known whether it was protective to new infection or not. Among the risk factors, sex, age, breed and woreda were found to be the potentially correlated to the occurrence of IBD in the study area. Thus, further study has to be conducted to decide whether the chicken population in the study area needs vaccination or not.

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Table1. Seroprevalence of IBD with Sex

Sex	Total examined	+Ve result	χ2-value	P-value
Male	290	243(83.8%)	**0.704**	**0.401**
Female	262	215(82.1%)		
Total	**552**	**458**		

Table2. Seroprevalence of IBD with Age

Age	Total examined	+Ve result	χ2-value	P-value
3-6 weeks	381	360 (94.5%)	**314.39**	**0.000**
0-3weeks	171	98(5.73%)		
Total	**552**	**458**		

Table3. Seroprevalence of IBD with Breed

Breed	Total examined	+Ve result	χ2-value	P-value
Local	436	353 (81.0%)	**314.39**	**0.000**
Cross	116	105 (90.5%)		
Total	**552**	**458**		

Table4. Seroprevalence of IBD with Woredas

Woredas	Total examined	+Ve result	χ2-value	P-value
Adama	203	164(75.3%)		
Modjo	184	141(76.6%)	**314.39**	**0.002**
Wenji	165	152(92.1%)		
Total	**552**	**458**		

Table5. Seroprevalence of IBD with Kebeles of the Districts

Kebeles	Total examined	+Ve result	χ2-value	*P-value*
Qobo Lixo	105	94 (89.2%)		
Saqa Kelo	123	101(82.1%)	**3.791**	**0.07**
Boko Shenan	116	85 (73.3%)		
H. Melkasa	132	110 (83.3%)		
Kella	76	68 (89.5%)		
Total	**552**	**458**		

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