Seroprevalence of Infectious Bursal Disease and Its Associated Risk Factors in Backyard Chickens in Selected Districts of Illubabor Zone, Oromia Regional State, Western Ethiopia

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Abstract: A cross-sectional study was conducted to assess the seroprevalence of infectious bursal disease virus and its risk factors in backyard chickens in selected districts of Illubabor Zone, western Ethiopia. A purposive sampling technique was applied for the selection of three districts and nine *kebelles* (the smallest administrative unit) of the study Zone, while simple random sampling was used for the collection of serum samples from individual chickens. A total of 384 serum samples were collected for the detection of antibodies against infectious bursal disease virus using ProFLOK® PLUS indirect enzyme-linked immunosorbent assay (ELISA). The identification of risk factors was conducted using a questionnaire survey. The overall seroprevalence of infectious bursal disease was found to be 46.61%. The prevalence was higher in young (58.61%) than in adult (17.12%) chickens and the difference was significant ($P<0.05$). Moreover, a higher but nonsignificant ($P>0.05$) seroprevalence of infectious bursal disease was observed in Halu district (48.1%), followed by Algesachi (47.0%) and Didu (44.4%). The majority of respondents didn`t have any awareness about infectious bursal disease. The knowledge about infectious bursal disease was significantly associated ($P < 0.05$) with marital status, location (districts), and education level of the respondents. The study revealed a very high seroprevalence of infectious bursal disease virus in backyard poultry production, which indicates a circulating virus in the area. In the study districts, a good management system coupled with vaccination programs should be practiced to reduce the incidence of infectious bursal disease.

Keywords: *Backyard chicken, Indirect ELISA, Infectious bursal disease, Illubabor, Seroprevalence*

Introduction

In developing countries, poultry production plays a significant role in securing family nutrition and economic benefits. Ethiopia has a large population of chickens, with an estimated population of 40.6 million, from which 99% are raised in the backyard management system, while the rest are reared under an intensive management system (CSA, 2014).

In Ethiopia, management problems, predators, and diseases have considered as the main constraints to the poultry sector. Fron these, diseases are the major factors, which lead to 20 to 50% of mortalities in chickens (Kinung`hi *et al*., 2004; OIE, 2004). One of the most serious illnesses that can harm chickens in various parts of Ethiopia is infectious bursal disease (IBD). The disease is thought to have been introduced with the increased numbers of commercialized and privately owned poultry farms in the country, which ultimately results in a decline in the industry's production and productivity across the country (Zeleke *et al*., 2005).

Infectious bursal disease is caused by *Infectious bursal disease virus* (IBDV), which is a member of the genus *Avibirnavirus* of the family *Birnaviriidae*. The disease is characterized by its acute onset and highly contagiousness in young chickens (Aschalew *et al*., 2003). IBDV is a double-stranded ribonucleic acid virus (RNA) having two segments, i.e. A and B. The virus has the two serotypes, with serotype 1 to be the only pathogenic to chickens. It was documented that there appear to be viral strains with antigenic and pathogenic variants. Genetic mutations in the genome of the virus are the basis for the emergence of antigenic and pathogenic variant strains. Owing to the IBDV's resistance, the disease tends to recur in a succeeding flock (Butcher and Miles, 2003; Eterradossi and Saif, 2008).

Globally, infectious bursal disease is present in every major region that produces poultry. Considering the presence of IBD in over 95% of Office International des Epizooties (OIE) member countries, in 1995, it was estimated during the organization's 63rd general session that the disease has significant socio-economic significance (OIE, 1995). In Ethiopia, IBD has been reported since 2005, with an outbreak report in Bishoftu commercial poultry farms in 20 to 45 days old broiler and layer chickens, with a mortality rate ranging from 45 to 50% in different poultry houses and an overall mortality rate of 49.83% (Zeleke *et al*., 2005). Moreover, Mazengia *et al*. (2010) reported 21.7-29.4% seroprevalence in different districts of northwest Ethiopia. Despite the importance of IBD, there is a limited report on the prevalence and risk factors in the backyard chickens in Illubabor Zone. Therefore, this study was aimed to estimate IBD seroprevalence and to

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Published by Haramaya University, 2022

ISSN: 2616-8804 (Print); 2959-0531 (Online)

assess the risk factors for IBD occurrence in selected districts of Illubabor Zone.

Materials and Methods

Study Area Description

The study was conducted in Illubabor Zone of Oromia Regional State, Ethiopia which is located about 600 km from Addis Ababa (i.e. the country's capital city). Illubabor is located at a latitude of 7° 05` and 8° 45` N and longitude 33° 47` and 36° 52` E, altitude 1400-2000 meters above sea level (m.a.s.l.). The average annual rainfall in the area is 1800 mm. The average annual temperature of the area is 20.7^{O}C. Metu is the capital city of Illubabor Zone, which is located on the main road of the capital city to Gambella. It covers a total land area of 999,625 square kilometers. Algesachi, Didu, and Halu districts were selected purposively for this research based on the poultry population and report of high mortality of chickens in the area. Specific description of the selected districts in the study areas.

Halu district: It is found about 48 km from Metu and located at a latitude of 8^{o} and 9`0" N and longitude 35° 20` 0" E, with an altitude of 1670.00 m.a.s.l. In the area, there are estimated 47,763 cattle, 31,414 sheep, 6,885 goats, 4,308 equine, and 116,329 poultry population (CSA, 2014).

Didu district: It is found about 57 km from Metu and located at a latitude of 7° 07`22-442" N and longitude 035° 22`12-548"E, with an altitude of 1780 m.a.s.l. In the area, there are estimated 89,159 cattle, 24, 536 sheep, 9,018 goat, 17,115 equine, and 95,785 poultry population (CSA, 2014).

Algesachi district: It is found about 54 km from Metu and located at a latitude of 8° 38` and 46° 80"` N and longitude 35° 47` and 23° 150"` E, with an altitude of 1756 m.a.s.l. In the area, there are estimated 173,349 cattle, 207,359 sheep, 95,812 goats, 62,251 equine, and 701,562 poultry (CSA, 2014).

Study Population

The study chickens were unvaccinated and apparently healthy backyard chickens. A questionnaire was filled to collect data on vaccination status, age, sex, breed, origin of the chicken, and education status and knowledge of the respondents about the disease. The chickens were categorized into young (≤3 weeks) and adult (> 3 weeks), which is based on the development of the bursa of Fabricius, which determines the susceptibility of chickens to IBDV infection.

Study Design

A cross-sectional study was conducted from December 2021 to November 2022 to estimate the seroprevalence and assess the associated risk factors of infectious bursal disease in the study districts. The households were clustered based on chicken ownership and randomly selected from each PA.

Sampling Methods and Sample Size

Purposive sampling technique was employed to select districts and *kebelles* as the places had high poultry potentials and high mortality of chickens as reported by officials in the districts. Three districts (Halu, Didu and Algesachi) were selected from the Zone. From each district, three *kebelles* were selected. The sample size was calculated according to Thrusfield (2005) formula:

$$n = 1.96^2(Pexp)(1-Pexp)/d^2$$

Where, n= Sample size; Pexp= Expected prevalence, d= Desired level of precision.

Thus, taking 50% expected prevalence, with 95% confidence interval and 5% desired absolute precision, 384 chickens were included in the study. Simple random sampling was applied for sample collection from the chickens. For the questionnaire survey, the sample size was calculated according to the formula (i.e., $N= 0.25/(SE)^2$) of Arsham (2002). Thus, considering a 5% standard error, 0.05 precision level, and 95% confidence interval the sample size was calculated as 100. However, 120 interviewees were included to increase the accuracy.

Sample Collection

Using a 5 ml sterile disposable syringe having 11/4 needle size and a 22 gauge, about 2-3 ml of blood was drawn from the chickens' wing veins. Then the blood samples were placed horizontally at a 45-degree angle and allowed to clot for three to four hours at 4ºC. The separated serum was then transferred into a labeled sterile cryovial tube and transported to Bedelle Regional Laboratory for laboratory using a cold chain (i.e., ice box containing ice pack). After arrival, the sera in the tube were centrifuged at 1000rpm for clarification and then stored at -20ºC until tested for IBDV antibodies.

Laboratory Analysis

IDvet innovative diagnostic indirect ELISA kit (Louis Pasteure-Grabels, France) was used to detect the presence of anti-IBDV antibodies in the chicken serum. All the procedures and protocols were based on the kit manufacturers' recommendations and according to OIE (2004).

Questionnaire Survey

Information related to the chickens' attributes like breed, sex, age, origin of chicken, and status of vaccination were collected. Besides, information on owners' sex, owner age, educational level and experience in rearing chickens, recent introduction of new chickens and awareness of IBD were collected using a questionnaire format prepared for this purpose. Before data collection, verbal agreement was obtained from the respondents.

Data Management and Analysis

All the data collected were entered into MS Excel spread sheet 2019 before analysis. Data was analyzed using STATA version 14 (StataCorp, 2015) and descriptive statistics such as frequency and proportion was used to estimate the seroprevalence of IBD. Chi-square test was used to test the association of age, sex, breed and origin of chickens with the disease. In all the analyses, $P<0.05$ was set for significant association.

Results

Overall Prevalence of Infectious Bursal Disease

Out of the total sera tested, the overall seroprevalence of IBD was 46.61% (179/384).

Risk Factors for Seroprevalence of Infectious Bursal Disease

Sex and age-wise seroprevalence of IBD is presented in Table 1. Out of sera examined, 46.9% male and 46.3% female chickens were positive for IBD and there was no statistically significance variation ($P>0.05$). The seroprevalence of IBD based on age was found to be 58.61% and 17.12% in young and adult age groups, respectively, with a statistically significant difference ($P<0.05$) between age categories.

Table 2 presents the prevalence of IBD based on chicken's breed and origin. The seroprevalence of IBD was found to be 47.2% and 45.5% in local and crossbreed chickens, respectively. There was no statistically significant difference ($P>0.05$) between breed categories. The seroprevalence of IBD based on origin was found to be 48.9% and 43.5% in existing and newly introduced chickens, respectively, but there was no statistically significant difference ($P>0.05$).

Among the studied districts (Table 3), the highest prevalence was recorded in Halu (48.1%) followed by Algesachi (47%), but there was no statistically significant difference ($P>0.05$). At *kebelle* level, IBD prevalence was highest in Halu Gamachis (70.2%). However, there was no statistically significant association ($P>0.05$) between the occurrence of IBD and studied *kebelles* (Table 3).

Table 1. Sex and age wise seroprevalence of infectious bursal disease in Illubabor Zone, western Ethiopia.

Variables	Categories	№ of animals examined	№ of IBD seropositive animals	χ2 - value	P-value
Sex					
	Male	181	85 (46.9%)	0.0165	0.898
	Female	203	94 (46.3%)		
Age					
	Young	273	160 (58.61%)	54.5904	0.0001
	Adult	111	19 (17.12%)		

IBD= Infectious bursal disease.

Table 2. Breed and origin wise seroprevalence of infectious bursal disease in Illubabor Zone, western Ethiopia.

Variables	№ of animals examined	№ of IBD seropositive animals	χ2 - value	P-value
Breed:				
Local	250	118 (47.2%)	0.0987	0.753
Crossbred	134	61 (45.5%)		
Origin:				
Home bred (existing flock)	223	109 (48.9%)	1.0958	0.285
Bought (newly introduced)	161	70 (43.5%)		

IBD= Infectious bursal disease.

Table 3. Districts and *kebelle* wise seroprevalence of infectious bursal disease in Illubabor Zone, western Ethiopia.

Variables	№ of animals examined	№ of IBD seropositive animals	χ2 - value	P-value
Districts:				
Halu	135	65 (48.1%)		
Didu	117	52 (44.4%)	0.0456	0.672
Algesachi	132	62 (47.0%)		
***Kebelles*:**				
Sedro	48	13 (27.1%)		
Halu Gamachis	37	26 (70.2%)		
Kersa	50	26 (52.0%)		
Gamachis	39	11 (28.2%)		
Lalo	40	21 (52.5%)	0.587	0.2
Gordomo	38	20 (52.6%)		
Chomoso	51	23 (45.1%)		
Uso Sege	45	21 (46.6%)		
Danbato	36	18 (50.0%)		

IBD= Infectious bursal disease.

Questionnaire Results

All (100%) of the respondents reported that chicken mortality was high in their locality area. Most of the respondents (83.3%) didn't have any awareness about the disease. In this study area, 70.8% of interviewed poultry owners had no formal education. The majority (66.7%) of respondents were females. Moreover, 75% of the respondents were married (Table 4).

Table 4. Socio-demographic information of study participants and knowledge about the disease (N=120).

Items	Variables	Number of respondents	Proportion
District	Halu	45	37.5%
	Didu	35	29.2%
	Algesachi	40	33.3%
Gender of respondent	Male	40	33.3%
	Female	80	66.7%
Marital status	Single	30	25%
	Married	90	75%
Educational background	Primary	25	20.8%
	Secondary	10	8.4%
	No	85	70.8%
Chicken mortality	Yes	120	100%
	No	0	0%
Owner's knowledge of the disease	Yes	20	16.7%
	No	100	83.3%
Newly introduced chicken	Yes	40	33.3%
	No	80	66.6%

Table 5 presents the analysis of factors associated with IBD awareness status in the study districts. Thus, 31.1% of respondents in Halu district were aware, while only 11.4% and 5.0% were aware from Didu and Algesachi districts, respectively. Based on educational status, 80.0% of those with secondary education were aware of the disease, while only few (5.9%) with no formal education had awareness about the disease. The district, marital status, and educational status of respondents education status was significantly associated with the awareness of the disease ($P<0.05$).

Table 5. The association of socio-demographic information of participants with knowledge of disease (N=120).

Demographic status of the respondent	Number of interviewed respondents	Number (%) of respondents with awareness	P-value
Sex:			
Male	40	8 (20.0)	0.488
Female	80	12 (15.0)	
Marital status:			
Single	30	9 (30.0)	0.023
Married	90	11 (12.2)	
Districts:			
Halu	45	14 (31.1)	
Didu	35	4 (11.4)	0.003
Algesachi	40	2 (5.0)	
Education status:			
Primary	25	7 (28.0)	
Secondary	10	8 (80.0)	<0.001
No	85	5 (5.9)	

Discussion

The current overall IBD seroprevalence (46.61%) is comparable with the report of Tsai and Lu (1993) in Taiwan (45%) and Singh and Dhawedkar (1992) in India (46.2%), whereas, it is higher than that reported by Mahasin (1998) in Sudan (30.7%) and Mushi *et al.* (1999) in Botswana (30%). However, the present finding is lower than previous findings from Ethiopia, such as 75% in North Gondar and West Gojjam (Kassa and Mola, 2012); 82% in central Oromia (Zeryehun and Fekadu, 2012); 76.6% in districts of Oromia Regional State (Degefa *et al.*, 2010); 76.3% in East Shoa Zone (Reta, 2008); 93.3% (Zeleke *et al.*, 2005); and 100% (Woldemariam and Wossene, 2007). Moreover, a higher prevalence (82.5%) was reported in northern Tanzania (Swai *et al.*, 2011). These variations could be due to the differences in environmental and management systems. In addition, in the present study, the chickens were managed under poor conditions,

because the owners gave little attention to backyard chicken production.

The present study revealed that seroprevalence of IBD was slightly higher in local (47.2%) than crossbreed (45.5%) chickens, but without significant variations (P>0.05). This might be due to the reason that chicken were allowed to scavenge in similar environment, which is supported by previous findings of Degefa *et al.* (2010); Jenbreie *et al.* (2013); and Zeryehun and Fekadu (2012) from Ethiopia.

The seroprevalence was higher in younger (58.61%) than adult (17.12%) chickens and the variation was statistically significant (P<0.05). The bursa of Fabricius attains its maximum size when chickens are aged between 0 to 3 weeks and makes them more susceptible to IBDV, because it is the site of multiplication for the virus (Eterradossi and Saif, 2008). Moreover, Jenbreie *et al.* (2013) reported higher seroprevalence in young age group (86.6 %) than adult of age (72 %). The present finding also agrees with the reports of Singh and Dhawedkar (1992), who reported that the prevalence was higher in 7-11 weeks old chickens (61.82 %) than above 22 weeks of age (3.92 %). Furthermore, Hitchner (1978) showed chickens between 2 and 7 weeks of age had higher susceptibility. In the course of IBDV infection, specific antibodies become detectable after 2 weeks and reaching a peak at 4 weeks (Hirai *et al.*, 1972).

In the present finding, the seroprevalence was numerically higher (P>0.05) in males (46.9%) than in female (46.3%) chickens, which is in line with previous studies in Ethiopia (Degefa *et al.*, 2010; Jenbreie *et al.*, 2013). This absence of significant association between the sexes is supported by the fact that there are no variations in exposure to IBDV in the field conditions.

From the studied districts, the seroprevalence was highest in Halu (48.1%) followed by Algesachi (47%). The prevalence and spread of IBDV could be affected by variations in management systems such as poor sanitation, exposure to adverse conditions and wild birds, nutritional factors and contact across villages (Smith, 1992). IBD can easily spread and persist in indigenous chickens since it is easy for hens from different places to come into contact with one another at open-air markets before being returned to their own localities. There is no specific environmental situation that can prevent or modify the occurrence of the disease (Berg, 2000). Production systems, types of strains, and environmental and management factors are also reported to be associated with the occurrence of the disease (Jenbreie *et al.*, 2013).

From the 120 interviewed respondents, 100% indicated that there was high chicken mortality in their area. Moreover, most of the respondents (83.3%) lack knowledge about the disease. The knowledge of respondents about the disease was significantly associated (P<0.05) with the district, marital status, and educational status of the respondents. The variation in educational status across studied districts could influence the level of awareness and attentions given towards IBD.

Conclusion

The present study revealed a high seroprevalence of infectious bursal disease in the study districts. This high prevalence of IBD might influence the income of producers from chicken in the study area. Moreover, the presence of IBDV-specific antibodies in non-vaccinated chickens indicates that the backyard chickens were exposed to the virus in the field. The disease has a significant association with age of the chickens and majority of the respondents lack knowledge about the disease. Thus, a participatory immunization program in backyard chickens of rural areas should be practiced. Additional research using molecular methods are needed to characterize the circulating strain of IBDV in the Zone.

Acknowledgments

The authors acknowledged Wollega University, School of Veterinary Medicine and Bedelle Regional Veterinary Laboratory for their contribution to this research work.

Conflict of Interests

The authors declare that they have no competing interests.

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