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Full Research Article

DETECTION OF INFECTIOUS BURSAL DISEASE VIRUS (IBDV) ANTIBODIES IN BACKYARD POULTRY BY USING INDIRECT ENZYME LINKED IMMUNOSORBENT ASSAY

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Abstract

Infectious bursal disease virus (IBDV) causes infectious bursal disease in poultry and poses a major challenge to the poultry industry globally. This study aimed to measure seroprevalences and so detect exposure to IBDV in backyard poultry in the selected zone of Horro Guduru Wollega. A cross-sectional study was conducted from January 2021 to November 2022. Blood samples were collected for serum extraction from 384 backyard chickens in the Horro and Horro Bulluq districts. IBDV antibody detection was conducted using an indirect ELISA serological diagnostic test. Questionnaires assessed poultry owners' knowledge and health/hygiene management practices regarding the disease. The over all seroprevalence of IBDV was 14.84%. Significant variations in seroprevalence were seen based on district, bird age, bird sex, and flock size. Limited owner experience (just 1-3 years), disposing of carcasses in pits, and poor hygiene on the backyard premises were associated with higher IBDV seroprevalence. In conclusion, IBDV seroprevalence was linked to chicken management practices. Recommendations include improving poultry management among owners to control

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IBDV. The study indicates backyard poultry in the region have considerable IBDV exposure, and control should focus on improving management practices identified as high-risk, such as pit disposal of carcasses and poor hygiene.

Key Words: backyard chickens, Infectious bursal disease, risk factor, seroprevalence

INTRODUCTION

Poultry production plays a significant role in the economy and livelihoods of many developing countries (Alders et al., 2014). In Ethiopia, the total chicken population is estimated to be 57 million, of which 78.9% are indigenous, 12% exotic and 9.1% hybrid breeds (CSA, 2021). Backyard poultry keeping is an integral part of the farming system and provides income and high-quality protein to rural communities (Dessie & Ogle, 2001). However, infectious diseases remain a major constraint to improved productivity.

Infectious bursal disease (IBD), also known as Gumboro disease, caused by the highly contagious infectious bursal disease virus (IBDV) (Mili et al., 2022), is an important viral illness affecting young chickens worldwide. The illness was first recognized in 1962 in Gumboro, Delaware. IBDV is a double-stranded RNA virus in the family Birnaviridae (Julia, 2023). There are two IBDV serotypes, with serotype 1 being responsible for disease in chickens. Strains within serotype 1 exhibit antigenic variation, largely due to antigenic drift but also from genome recombination.

IBDV causes an immunosuppressive disease in young chickens aged 3-6 weeks (WOAH, 2024). IBDV targets the bursa of Fabricius, infecting the actively dividing B lymphocytes in immature chickens. This leads to morbidity, mortality, and immunosuppression. The immunosuppression makes chickens more susceptible to other infections and reduces vaccine efficacy against other diseases. By impairing B cell development and antibody production, IBDV infection of the bursa of Fabricius causes significant immune system damage in young chickens (Dey et al., 2019). Studies show IBDV continues to be an endemic threat globally due to emergence of variant strains (Mazengia et al., 2008). The virus spreads through fecal-oral transmission and has a short incubation period of 2-3 days (Van den et al., 2000). Various factors influence the severity of IBD outbreaks, including strain pathogenicity, flock susceptibility, the presence of other pathogens, and environmental stressors (Abdeta et al., 2022). Although IBD is considered one of the most important poultry diseases in Ethiopia, there have been limited epidemiological studies, especially in backyard systems (Chaka et al., 2013). This gap in evidence limits the design of effective prevention and control programs. Therefore, this study aimed to determine the seroprevalence and associated risk factors of IBDV in backyard chickens in and around Shambu town, Horro Guduru Wallaga zone, Oromia, Ethiopia, in order to generate evidence to support disease control efforts. The specific objectives were to: 1) determine the seroprevalence of IBDV in backyard chickens in the study area, and: 2) identify potential risk factors associated with IBDV seropositivity in the studied chicken flocks.

MATERIALS AND METHODS

Description of Study Area

The study was conducted in and around Shambu town, located in Horro Guduru Wallaga zone of Oromia region, Ethiopia. The zone has a poultry population of 472,374 (CSA, 2021). The study districts were Horro and Horro Bulluq, comprising highland, mid-altitude, and lowland agro-ecological zones. The climate has three seasons: main rainy season from June-October, dry season from November-February, and short rainy season from March-May (Horro Bulluq's Socio-Economic Profile, 2014).

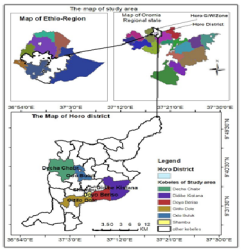

Figure 1: Map of the Study Area

Study Population

The study population was local chickens in backyard systems. The chickens were able to scavenge, but lived with households and other livestock. Breeds were Horro, exotic SASO T-44, and Bovans Brown. The chickens were categorized into three age groups according to Magwisha et al. (2002). Chickens over 12 months old were considered adult. Chickens between 6-12 months old were categorized as growers (young). Chickens under 6 months old were identified as chicks. The ages were estimated based on physical characteristics of each chicken, such as crown size, spur length, and flexibility of the xiphoid cartilage. Information collected included sex, breed, management, origin, hygiene, production system, and flock size.

Study Design

A cross-sectional study design was implemented from January to November 2022 to determine IBDV seroprevalence and associated risk factors in the backyard poultry population.

Sample Size Determination and Sampling Method

The sample size was determined using the formula by Thrusfield (2007) for an expected seroprevalence of 50%, precision of 5% and confidence level of 95%. This

gave a sample size of 384 chickens. A simple random sampling method was used to select chickens from different flocks in the study districts.

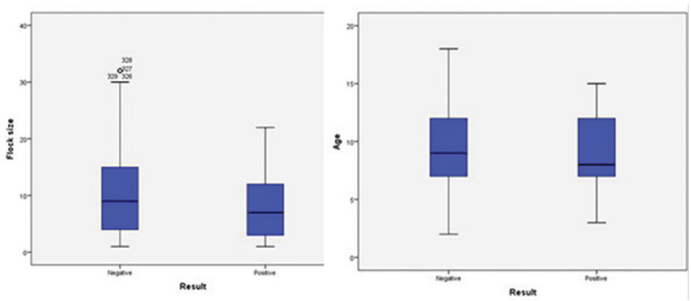

Figure 2: Relationships between (A) flock size and result, and (B) age and result

Questionnaire Survey

A survey was conducted of 100 randomly selected respondents from households with backyard chicken flocks. The sample size was determined using the Arsham (2007) formula to achieve a 5% standard error. Participants completed a semi-structured questionnaire investigating factors associated with IBDV infection in backyard flocks, such as owner education level, poultry-keeping experience, biosecurity practices like isolation of new birds, and carcass disposal methods.

Ethical Approval and Participant Consent

The study received ethical clearance from the Wallaga University Ethical Review Board under letter reference number WURSVM140/2022, approving the study protocol, animal handling procedures, and informed consent process. Informed written consent was obtained from all human participants before their enrollment in the study. Consent for collecting blood samples from animals was obtained from the animal owners. Standard veterinary practices were followed during the blood collection to ensure animal welfare.

Blood and Serum Collection

Approximately 2-3mL of blood was collected from wing veins of each chicken using sterilized 23 gauge needles and 3mL syringes at a 45° horizontal angle (n=384). Serum was separated by overnight incubation at room temperature. Serum samples were transported in a cold chain at 4°C to the laboratory.

Serological Testing

Serological testing was conducted using a commercial kit specific for IBDV antibody detection (ProFLOKIBV, USA) and following the manufacturer's instructions and WOAH guidelines (WOAH, 2024).

Data Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version IBM SPSS Statistics (Version 27). Descriptive statistics, chi-square tests, and multivariate logistic regression were applied. Associations were quantified by odds ratios at 95% CI and $p<0.05$ significance.

RESULTS

The overall seroprevalence of IBDV across the two districts of Horro and Horro Bulluq were 14.8%, with 57 positives out of 384 chickens tested. The seroprevalence differed significantly between the districts, with Horro having a seroprevalence of 18.7% (37/198) compared to 10.8% (20/186) in Horro Bulluq. The seroprevalence of antibodies against infectious bursal disease virus (IBDV) in backyard chickens varied widely depending on the origin of the flock (peasant association). Specifically, seroprevalence ranged from 3.3% in the Shambu 01 peasant association to 35.4% in the Gitilo Dale peasant association. This indicates that geographic factors may influence IBDV exposure rates in backyard poultry in this region. Additionally, seroprevalence was significantly associated with flock size, as large flocks were highly affected (29.2% seroprevalence), but small flocks much less so (2.4% seroprevalance). Origin did not have a significant impact, as home- and market-sourced chickens had similar seroprevalences of 14.2% and 14.8%, respectively (Table 1).

Table 1. Seroprevalence of infectious bursal disease virus by study district, flock size, and bird origin

Variable	Category	No. of Tested	No. of Seropositive	Seroprevalence	Pearson Chi-Square	P-Value
Districts	Horro	198	37	18.7%	4.776	0.032
	Horro Bulluq	186	20	10.8%		
Peasant Association	Dacha Caabir	83	10	12.0%	24.806	0.000**
	Didibe Kistana	60	12	20.0%		
	Doyo Bariso	30	5	16.7%		
	Gitilo Dale	48	17	35.4%		
	Oda Bulluq	103	10	9.7%		
	Shambu 01	30	1	3.3%		
	Shambu 02	30	2	6.7%		

Continuation of the table 1						
Flock size	Small (<8)	206	5	2.4%	41.845	0.007
	Large (>8)	178	52	29.2%		
Origin	Home	21	3	14.2%	0.005	0.620
	Market	363	54	14.8%		
Total		384	57	14.8%		

** =Highly significant

Younger chickens had higher IBDV seroprevalence (20.4%) compared to older adult chickens over 12 months of age (3.9%), indicating age is a significant risk factor (OR=10.4, p=0.000). Female birds also had higher seroprevalence (23.3%) than males (5.1%), showing female birds were at greater risk of contracting this virus (OR=2.948, p=0.027). Large flocks had much higher seroprevalence (29.2%) than did smaller flocks (2.4%), revealing flock size is a major risk factor (OR=43.4, p=0.000). In summary, the analysis identified young age, female sex, and large flock size as significant risk factors for higher IBDV seroprevalence in the studied chicken populations, while breed did not have a significant impact (Table 2).

Table 2. Multivariate Regression Analysis of seroprevalence of infectious bursal disease virus in association with animal-related risk factors in the study area

Risk Factors	Category	No. of examined	No. of positive	Prevalence	OR	SE	PV	95% CI
Age	Young	255	52	20.4%	10.402	.606	.000	3.170-34.130
	Adult	129	5	3.9%				
Sex	Male	178	9	5.1%	2.948	.487	.027	1.134-7.663
	Female	206	48	23.3%				
Breed	Cross	37	8	21.6%	1.748	.517	.280	.635-4.812
	Local	347	49	14.1%				
Flock	1-8	206	5	2.4%	43.437	.514	.000	15.859-118.974
	> 8	178	52	29.2%				
Total		384	57	14.84				

A correlation analysis was conducted using data from the 384 birds to assess the relationship between flock size and age of poultry (Table 3). The results showed a statistically significant negative correlation between flock size and age (r=-0.219, p=0.000). As flock size increased, the age of poultry tended to decrease, indicating that larger flocks were associated with younger birds. While the correlation was weak, it was highly statistically significant (p<0.01). This suggested that in the sampled poultry population, larger flocks tend to have younger average flock ages. Supporting this, a separate multivariate regression analysis (Table 2) identified young age and large flock

size as significant risk factors for higher IBDV seroprevalence in the studied chicken populations. Together, these analyses demonstrate that larger flock sizes are associated with younger birds, which contributes to increased IBDV infection risk.

Table 3. Correlations of flock size and age of poultry

		Age	Flock size
Age	Pearson Correlation	1	-.219**
	Sig. (2-tailed)	0.000	.000
	N	384	384
Flock size	Pearson Correlation	-.219**	1
	Sig. (2-tailed)	.000	
	N	384	384

The study included a total of 100 respondents to the survey, with 27 (27%) from Horro Bulluq district and 73 (73%) from Horro district. Most respondents were female (90% or 90 respondents) compared to just 10% (10 respondents) who were male. Over half of the respondents (54%, 54 respondents) were married, 34% (34 respondents) were single, 9% (9 respondents) were divorced, and 3% (3 respondents) were widowed. In terms of education, 55% (55 respondents) had no formal education, 21% (21 respondents) had completed school grades 1-4, 12% (12 respondents) had finished grades 5-12, and 12% (12 respondents) were educated at levels higher than grade 12. Regarding religion, 39% (39 respondents) were Orthodox, 40% (40 respondents) were Protestant, 4% (4 respondents) were Wakefata, and 17% (17 respondents) were Muslim. Occupations were nearly evenly split between farmers (47% or 47 respondents) and merchants (53% or 53 respondents). In Horro Bulluq, all 27 respondents were female, while in Horro 86% (63 respondents) were female and 14% (10 respondents) were male. Overall, the table provides the demographic profile of the survey respondents from the two districts using percentages and frequencies (Table 4).

Table 4. Demographic characteristics of the respondents in the study area

Variables	Categories	District		Total No. of Respondents
		Horro Bulluq	Horro District	
Age	Adult (18-65 years)	19 (70.4%)	57 (78.1%)	76 (76.0%)
	Elderly (above 65 years)	8 (29.6%)	16 (21.9%)	24 (24.0%)
Sex	Male		10 (13.7%)	10 (10.0%)
	Female	27 (100.0%)	63 (86.3%)	90 (90.0%)
Marital status	Single	10 (37.0%)	24 (32.9%)	34 (34.0%)
	Married	14 (51.9%)	40 (54.8%)	54 (54.0%)
	Divorced	3 (11.1%)	6 (8.2%)	9 (9.0%)
	Widowed		3 (4.1%)	3 (3.0%)

Continuation of the table 4				
Education level	No education	12 (44.4%)	20 (20.0%)	55 (55.0%)
	Grade 1-4	9 (33.3%)	6 (6.0%)	21 (21.0%)
	Grade 5-12	3 (11.1%)	9 (12.3%)	12 (12.0%)
	> Grade 12	3 (11.1%)		3 (11.1%)
Religion	Orthodox	10 (37.0%)	57 (78.1%)	39 (39.0%)
	Protestant	14 (51.9%)	16 (21.9%)	40 (40.0%)
	Wakefata	1 (3.7%)	10 (13.7%)	4 (4.0%)
	Muslim	2 (7.4%)	63 (86.3%)	17 (17.0%)
Occupation	Farmer	13 (48.1%)	24 (32.9%)	47 (47.0%)
	Merchant	14 (51.9%)	40 (54.8%)	53 (53.0%)

Respondents most commonly had 1-6 years (20% with 1-3 years; 12% with 4-6 years) of experience with the disease. Very few (6.6%) had >6 years' experience. Use of traditional medicine for sick chickens was low (2.4%), with most doing nothing (17.2%). No respondents reported using modern medicine. The uptake of vaccination against IBDV in the chickens was also low (4%) compared to non-use (13.3%). Most respondents implemented irregular hygiene (17.2%) measures rather than regular hygiene (2.4%) measures. Small flocks of 1-8 birds were less common (2.4%) than large flocks of >8 birds (60%). Water sources were predominantly spring (15%) and pond (10%) rather than tap water (0%). Housing the birds together with the family (8.5%) was more common than caged housing (22.2%). Carcass disposal was largely by throwing into a pit (17.2%) rather than burial (0%). Few respondents practiced isolation (quarantine) of sick birds (4.8%) compared to non-isolation (15.5%). Overall, the data indicates small-scale production with limited disease prevention or control measures (Table 5).

Table 5. Knowledge assessment of poultry production and management systems

Characteristic Factors	**Category**	**No of Responders**	**Yes**	**Percentage**
Years of experience with the disease	1-3 years	60	12	20.0%
	4-6 years	25	3	12%
	> 6 years	15	1	6.6%
Type of medication preferred to treat sick chickens	Traditional	42	1	2.4%
	Modern	0	NA	N.A
	Do nothing	58	10	17.2%
Use of vaccine for disease prevention	Yes	100	25	25%
Implementation of hygiene measures	Regular	42	1	2.4%
	Irregular	58	10	17.2%

Continuation of the table 5

Source of water used	Tap water	0	0	0.0%
	Spring	20	3	15.0%
	Pond	80	8	10.0%
Bird housing (during night)	Cage	18	4	22.2%
	With family	82	7	8.5%
Carcass disposal practice	Buried	36	0	0.0%
	Thrown into pit	64	11	17.2%
Isolation (quarantine) of sick chickens	Practiced	42	2	4.8%
	Not practiced	58	9	15.5%

DISCUSSION

The current study found a 14.84% seroprevalence of IBDV antibodies in backyard chickens in Ethiopia. This is lower than was reported by Abdeta et al. (2022), Zegeye et al. (2015), and Lemma et al. (2019), with higher seroprevalences also reported in other parts of Ethiopia (Jenbreie et al., 2012; Chaka et al., 2012). The seroprevalence differences are likely due to variations in diagnostics, breed, environment, management, and disease awareness (Chakma, 2015; Hailu et al., 2009, 2010; Kassa & Molla, 2012; Mazengia et al., 2009; Reta, 2008; Shiferaw et al., 2013). The difference in seroprevalence between this study and previous ones is likely due to variations in sample types and serological tests used. For example, Jenbreie et al. (2012) tested serum samples collected during and after IBD outbreaks, while a less sensitive ELISA kit was used by Camilotti et al. (2016) compared to that used in the current study.

Younger chickens showed a higher seroprevalence than older chickens, agreeing with Abdeta et al. (2022), and which is potentially related to immunological differences between birds of different ages (Tippenhauer et al., 2013). Female birds had a higher seroprevalence than males, with the greater infection risk in females possibly relating to reproductive demands (Yosef et al., 2021; Abdeta et al., 2022).

A lower seroprevalence occurred with greater owner experience, while a higher seroprevalence was found for less owner experience (Abdeta et al., 2022), showing owner experience improves IBD control.

Proper disposal (0%) versus discarding carcasses into a pit (17.2%), and regular (2.4%) versus irregular (17.2%) hygiene measures were also associated with lower seroprevalences, highlighting the importance of good biosecurity practices. No statistically significant differences were observed among chicken breeds. However, this study found indigenous chickens had a numerically lower seroprevalence than did cross breeds, consistent with Jenbreie et al. (2012) and Zeryehun and Fekadu (2012). This could reflect innate resistance in indigenous chickens from long-term

virus exposure and co-evolution, versus stricter biosecurity for imported exotic breeds that reduces virus exposure. Selection for productivity traits could also increase exotic breed susceptibility.

CONCLUSION

In conclusion, the 14.84% seroprevalence indicates backyard chickens in the studied area are likely exposed to IBDV. Flock size and breed were important risk factors. Producers lacked knowledge of IBD. Proper management and bird vaccination are needed to reduce IBD incidence. Authorities should address IBDV to improve chicken production. Age, sex, flock size, owner experience, carcass disposal method, and hygiene measures significantly impacted chicken IBDV seroprevalence. Control efforts should target high-risk groups of birds and improve biosecurity. Further studies on circulating IBDV strains are warranted.

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Authors' contributions

GB & YF collect data and prepared initial draft of the manuscript. CG conducted Serological Investigation, LY , EH, TR , DT, AF, HW, DO reviewed and contributed to sections and improvement of the manuscript. EH finalized the manuscript for submission.

Competing interests

The authors declare that they have no competing interests

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DETEKCIJA ANTITELA ZA VIRUS GAMBORO BOLESTI DOMAĆE ŽIVINE KORIŠĆENJEM INDIREKTNOG ELISA TESTA

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Kratak sadržaj

Virus infektivnog burzitisa (IBDV) izaziva Gamboro bolest živine i predstavlja značajan izazov za industriju živine širom sveta. Ova studija ima za cilj da izmeri seroprevlenciju i otkrije izloženost domaće slobodno gajene živine virusu Gamboro bolesti u izabranom području Horro Guduru Wallage. Sprovedena je studija od januara 2021. do novembra 2022. godine. Uzorci krvi su prikupljeni radi izdvajanja seruma od 384 pileta u distriktima Horro i Horro Bulluq. Detekcija antitela na IBDV je izvršena korišćenjem indirektne ELISA dijagnostičke metode. Upitnici su procenjivali znanje vlasnika živine i primenu zoohigijenskih mera u vezi sa bolešću. Ukupna seroprevlencija IBDV-a iznosila je 14,84%. Primećene su značajne varijacije u seroprevlenciji na osnovu distrikta, starosti i pola živine te veličine jata. Ograničeno iskustvo vlasnika (samo 1-3 godine), odlaganje leševa u jame i loša higijena na imanju bili su povezani sa većom seroprevlencijom IBDV-a. Seroprevlencija IBDV-a bila je povezana sa načinom držanja živine. Preporuke uključuju poboljšanje zoohigijenskih mera pri gajenju živine kako bi se kontrolisao IBDV. Studija ukazuje na to da domaća živina u ispitivanom regionu ima značajnu izloženost IBDV-u, a kontrola bi trebalo da se fokusira na poboljšanje primene zoohigijenskih mera koje su identifikovane kao visokorizične, poput odlaganja leševa u jame i loše higijene.

Ključne reči: slobodno gajena živina, Gamboro bolest, faktori rizika, seroprevalencija