



Seroprevalence, Isolation and Molecular Detection of Infectious Bronchitis in Backyard and Commercial Chickens in Central Gondar Zone, Northern Ethiopia

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Abstract

Infectious bronchitis (IB) is a highly contagious disease of the respiratory and urogenital tract of chickens, caused by infectious bronchitis virus (IBV), a member of the family Coronaviridae. Due to the serious infectious and transmission features of the disease mostly in the reproductive and respiratory systems it causes potential economic loss. Hence a cross-sectional study was conducted from February 2022 to June 2022 on chicken serum and tracheal swab samples from backyard and commercial farms in central Gondar zone, Ethiopia, to determine the seroprevalence of IB, associated risk factors and for isolation and molecular detection of the virus. A total of 384 blood samples were collected and tested by an indirect ELISA and Anti-IBV antibody positivity was noted in 92.19% (95% confidence interval (89 % -94.6 %) of the samples. Logistic regression analysis was performed to determine the impact of possible risk factors on seropositivity. Higher prevalence was noted in young chickens than in adults ($p < 0.05$) and in exotic breeds than in local breeds ($p < 0.05$). Though the difference was not statistically significant ($p > 0.05$), higher prevalence was obtained in dual purpose chickens (93.75%) than in layers (92.17%) and broilers (90.98%). Higher prevalence was also noted in females (92.7%) than in males (90.98%) and in intensively managed chickens (93.39%) than in extensively managed chickens (90.69%) with $p > 0.05$. Conventional RT-PCR test was also performed for the molecular detection of virus. The test was done on 52 tracheal swab samples collected from intensive and backyard unvaccinated chickens that were pooled in to 26 samples. Accordingly, 3 (11.54%) of the 26 pooled samples were IBV positive. The result showed that this was the first molecular evidence found in the study area. The seroprevalence of the disease in this study was very high for all age groups, breed types, and farm types. The risk factors mentioned and the management methods may have raised the likelihood of infection and the prevalence of the disease. Vaccination and biosecurity measures are advised to manage the disease. Identification and characterization of persistent IBV serotypes that are present in the field is also recommended to manage the disease.

Keywords: IBV; Seroprevalence; Molecular detection; Central Gondar; Virus Isolation

Abbreviations: IB: Infectious Bronchitis; SPF: Specific pathogen-free; PBS: Phosphate Buffer Saline.

Introduction

Background of the Study

The total chicken population in Ethiopia is estimated to be 56.5 million. However, the economic contribution of the sector is not still proportional to the huge chicken numbers, attributed to the presence of many productions, reproduction and infrastructural constraints. Diseases and predators are known to be the major causes of losses in the country. Newcastle disease accounted for the largest proportion of overall flock mortality to be 57.3% followed by fowl pox 31.6%, coccidiosis, 9.4% and predator loss 1.7% [1].

Infectious bronchitis virus causes acute highly contagious viral disease of chicken known as infectious bronchitis, which is characterized by lesions in the reproductive, urogenital, and respiratory organs [2].

Chickens of all ages can contract the disease, although young chickens are more vulnerable because resistance builds with age. Due to poor performance, lower egg output and quality, and mortality that can be severe in the presence of nephropathogenic strains or when secondary infections emerge, the condition generates significant economic losses in the chicken industry following avian influenza; it is believed that IBV infection causes the third-highest losses among all livestock diseases [3].

The virus is a member of the Coronaviridae family and its 27.6 kb single-stranded positive sense RNA genome has the following genomic arrangement and: fifty untranslated regions 3'UTR: 1a/1ab S 3a 3b E M 5a 5b. While the non-structural viral proteins are encoded by two polyproteins, the structural viral proteins include the membrane (M), small membrane (E), nucleoprotein (N), and spike (S). The spike protein, among others, is the subject of extensive research due to its genetic diversity and biological function [4].

Following the discovery of the first IBV serotype in USA in 1931, the virus was detected in other US states, and a large variety of IBV genotypes and serotypes were described globally [5]. Infectious bronchitis is still a problem for the poultry industry despite global attempts to manage it thus far, mostly because of IBV's large genetic diversity, rapid generation time, and high mutation rate. The effectiveness of natural or vaccination immunity is hampered by the ongoing emergence of genetic diversity and the selection process among IBV variants [6].

In Africa, IBV is one of the most important viral diseases that threaten chicken production. It was first reported in Africa in 1950 in Egypt from birds with respiratory symptoms. The disease is the most prevalent viral respiratory infections of chickens in the continent and is widespread in both vaccinated and unvaccinated poultry birds and is regarded as an epidemic virus [7]. The disease is common in Ethiopia though its prevalence and type of strains has not been well studied in the present study area [8].

Objectives of the Study

General objectives: This study is aimed at determining the seroprevalence of infectious bronchitis virus antibody and to detect infectious bronchitis virus antigen of poultry in central Gondar zone.

Specific objectives: To determine the seroprevalence of infectious bronchitis in chickens within the study area

- To isolate and detect infectious bronchitis virus in poultry in the study area
- To study some associated risk factors

Statement of the Problem

Infectious bronchitis is one of the most serious viral infections threatening chicken farming in Africa. Despite reports of respiratory disorders in chicken from all production systems in Ethiopia, there is a paucity of reports on infectious bronchitis (IB). Accordingly, there is only one report about IB seroprevalence which indicates a seroprevalence of 24.6% [9] and no published evidence on molecular detection of the virus in northern Ethiopia in general and the study area in particular. Furthermore, the disease is a poorly studied condition in Ethiopia since it is frequently masked by other infections such as Newcastle disease and other respiratory diseases.

Research Questions

- What is the seroprevalence status of infectious bronchitis in the study area?
- What are the associated risk factors for the occurrence of infectious bronchitis?
- Is infectious bronchitis virus circulating in the study area?

Justification of the Study

Chickens in Ethiopia are not vaccinated against infectious bronchitis especially in rural areas where birds are mostly managed semi-intensively with little or no veterinary care and are left to search for food for most of the day. This practice promotes the rapid spread of the disease. Although

there are some published evidences on the sero-prevalence of the disease with variable figures across the country, there is a limited information on the seroprevalence and there is no information on molecular detection of the virus in both commercial and rural scavenging poultry in Central Gondar Zone. In order to prevent and control the disease by implementing vaccination and biosecurity measures, it is essential to identify the occurrence of the disease in the study area. Therefore; the present study was performed to determine the prevalence of the disease and to detect the existence of infectious bronchitis virus circulating in the study area.

Ethical Approval

Ethical clearance was obtained from the Ethics Committee of the National Veterinary Institute of Ethiopia. Accordingly, handling of the study animals throughout the study period was done based on the guideline of the institute. The poultry owners provided their permission and authorization for the collection of the samples.

Materials and Methods

The Study Area

Amhara Regional State is one of the largest state in Ethiopia with a large poultry population. The Central Gondar zone is located in the Amhara National Regional State, and its principal city, Gondar, is 727 kilometers from Ethiopia the capital, Addis Ababa (Figure 1). Geographically, the area lies between 12.3° and 13.38° N latitudes and 35.5° and 38.3° E longitudes. Central Gondar zone is located between 528 and 4620 meters above sea level. The average annual rainfall ranges from 880 mm to 1772 mm, with a monomodal distribution and temperatures ranging from 10°C to 44.5°C [9]. The overall population of central Gondar Zone is 2,642,114, with 2,204,225 (88.66 percent) living in rural villages that rely on agriculture for their livelihood. As a result, the study region is a significant source of livestock that contributes to the country's growth and domestic production. This area houses around 737,713 sheep, 1,479,366 goats, 3,234,012 cattle, 392,546 donkeys, 12,252 mules, 39,178 horses, and 3,310,498 chickens [10].

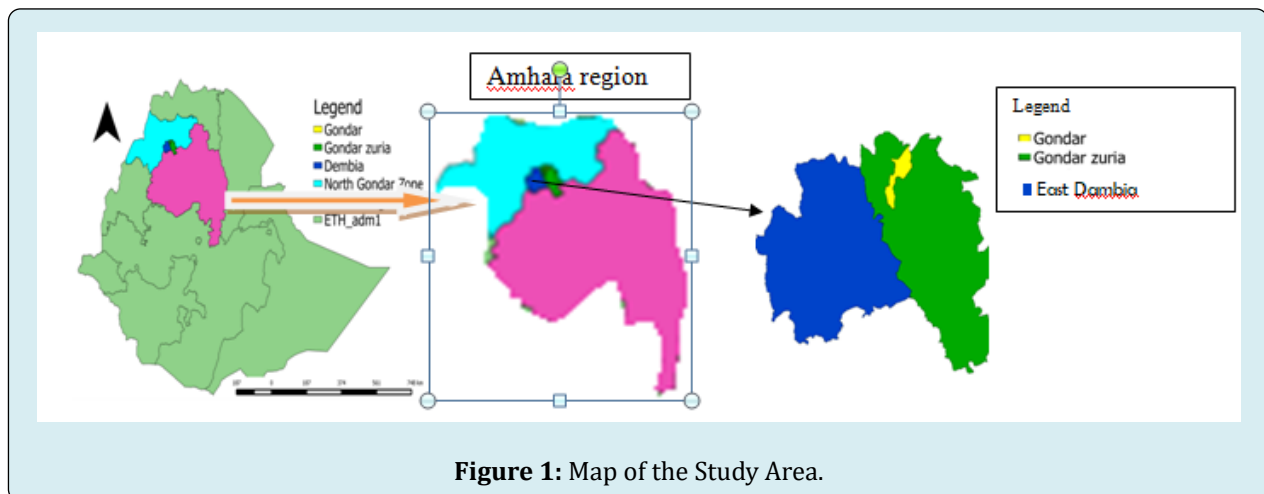


Figure 1: Map of the Study Area.

Study Design and Sampling Technique

Cross-sectional study design was used in this study. For district selection; a simple random sampling was utilized as a sampling strategy. A simple random sampling technique was also used to select poultry farms and individual chickens. Before blood and tracheal swab samples were taken, potential risk factors such as flock history (age, purpose of production, sex, breed, and method of production) and vaccination history in general were noted.

Study Animals

Because maternal antibodies are expected to diminish within three weeks of life, chickens were sampled when

they were above three weeks old. Exotic and local breeds of chickens were used in this study. Blood samples were collected from apparently healthy chickens and tracheal swab samples were collected from diseased chickens for RT-PCR analysis.

Sample Size Calculation

The sample size for the study was calculated using Thrusfield's formula [11], with a 95% confidence interval of 5% desired absolute precision and a prevalence of 24.61% from previous studies. Thus, 285 chickens were required for this study. However, to improve the precision of the study the sample size was expanded to 384.

$$N = \frac{1.96^2 P_{\text{exp}} (1 - P_{\text{exp}})}{d^2}$$

Where N= required sample size, P_{exp}= expected prevalence
d² =desired absolute precision. z=1.96, p=0.5 and d=0.05

Blood Sample Collection

Two to three ml blood sample was collected from unvaccinated chickens in different farms from the wing vein. To clot the blood, the collection tubes were kept slant for 24 hours at room temperature. Then sera were collected and placed into 2ml tubes and frozen at 20°C until they were processed at National Veterinary Institute of Ethiopia.

Swab Sample Collection and Preparation

Fifty two chickens that appeared to be diseased in total were chosen at random to have tracheal swab samples prepared for PCR analysis. Each swab was placed in a PBS (Phosphate Buffer Saline) containing antibiotic filled sterile tube and transported under cold chain and kept at -20°C in University of Gondar Veterinary Microbiology Laboratory. Then the samples were transported to the National Veterinary Institute of Ethiopia for processing under cold chain and kept under -80°C until processed. Then the samples were taken out from -80°C and thawed for a few minutes. Then in order to make it easier to remove the contents from the swab head, the swab samples from each flock were vortexed and samples from the same farm were pooled into one that makes a total of 26 samples which were then made ready for injection in to specific pathogen-free (SPF) eggs.

Virus Isolation

Specific pathogen-free (SPF) embryonic eggs were used in the viral isolation process. 0.2-0.3 ml of sample supernatant was inoculated into the allantoic cavities of 11day old embryos and no sample were inoculated in to the controls. Eggs were candled every day for four days with mortality occurring within the first 24 hours being regarded as nonspecific. After 96 hours of infection, all of the eggs allantoic fluids were collected and used to infect another egg groups up to three passages. Embryonic deaths after 24 hours of infection were observed and recorded. Infective allantoic fluids were collected from the third passage and kept at -20°C and processed by RT-PCR for molecular detection.

Serological Analysis

According to the manufacturer's recommendations, an indirect ELISA (ID Screen® Infectious Bronchitis Virus

Indirect) was performed to detect antibodies against IBV in chicken serum. The test is designed to detect IBV specific antibodies that are conserved across serotypes, allowing all IBV types to be detected. Four 96-well plates (precoated with purified viral antigen), positive and negative IBV antibody control sera, concentrated conjugate (10 xs), dilution buffer 14, dilution buffer 3, washing buffer (20 x), substrate solution, and stop solution were used in the test in accordance with the manufacturer's instructions. The microplates were examined using an ELISA micro plate reader at a wavelength of 450 nm. The ELISA sample to positive ratio for each serum determined the interpretation of the data. The percentage of positive sera among the total number of examined sera was used to calculate IBV prevalence.

The unknown test samples were evaluated Parallel to positive and negative controls,. Using a precoated ELISA plate and ready-to-use reagents, all conditions were standardized according to kit manufacturer specifications. The test was valid if the mean OD value of the positive control was greater than 0.250 and when the ratio of the positive and negative control mean values is greater than 3. The ELISA sample-to-positive (S/P) ratio for each serum was used to evaluate the results. Serum samples with an S/P value greater than 0.2 were considered positive.

$$S/P \text{ ratio} = \frac{OD_{\text{sample}} - OD_{\text{NC}}}{OD_{\text{PC}} - OD_{\text{NC}}}$$

Where S/P is the sample to positive ratio; OD is the optical density; OD_{NC} is the optical density of negative control; and OD_{PC} is the optical density of positive control.

RNA Extraction

RNA was isolated by using the Qiagen RNeasy Mini Kit protocol. Based on the this protocol 350µl of RLT lysis buffer was added to 350 µl sample aliquots to bring them to room temperature, and were mixed by pipetting. Next, 350 µl of 70% ethanol was added, and they were again mixed by pipetting. The sample mixture was then poured into an RNeasy spin column and centrifuged for 30 seconds at 13000 rpm. The flow through liquid was then discarded. After centrifuging RNeasy spin column for 30 seconds at 13000 rpm with 700 µl of buffer RW1, flow through liquid was discarded. Then 500 µl of RPE buffer was added in to RNeasy spin column and centrifuged at 13000 rpm for 2 minutes. After discarding the liquid flow, the column was put in a fresh 2 ml tube and centrifuged for 1 minute at 13000 rpm. The column was then put into a fresh 1.5 ml tube. Following the addition of 40 µl of RNase-free water, the column's membrane was centrifuged at 13000 rpm for one minute. The water containing RNA was then removed from the tube in an effort to recover as much of it as feasible. The membrane was then re-added to, and

it was centrifuged for 2 minutes at 13000 rpm. To keep the RNA until usage, the tube was finally closed.

RT-PCR Analysis

A standard One Step RT PCR Kit was used to test the pools for the presence of IBV by amplifying 466 bp hypervariable region of the S1 gene. Briefly, 1X reaction mix, 2µl of forward primer 5' XCE1(CACTGGTAATTTTCAGATGG)3' and 2 µl of reverse primer 3'XCE2 (CCTCTATAAACACCCTTGCA)5', primer pair, and one step RT PCR 1µl enzyme mix were combined with 5µl of isolated RNA. 5x-Q solution 5 µl, 5x-RT PCR buffer in 5µl, 1µl of 10- mM dNTPs mix, and then 4 µl of RNA-free water were added to make a total volume of 25µl. The thermal protocol that was chosen went as follows: 50°C for 30 min, 95°C for 15 min, 1 cycle, followed by 94°C for 30 sec, 55°C for 1 min, and 72°C for 1 min for 35 cycles. A final extension step lasting five minutes at 72°C was also carried out. Then 1.5 percent agarose gel was used to mix 3 grams of agarose and 200 ml of 1xTAE buffer and kept for two minutes in the microwave then take out of the microwave for cooling. After cooling down to room temperature for 5 minutes, 4 µl of Gel Red was added in to the gel and left for 20 minutes. Then 10 µl PCR product that had 4µl of loading dye added to it was then loaded into each well along with a negative control and a positive control. Finally, 10µl of a molecular marker (ladder) starting at 100 bp was added. After that, electrophoresis was carried out for 1:20 hours at 120 volts, and the result was read under UV light. IBV results that are positive display 466 bp.

Data Analysis

The data collected from field level and laboratory investigation was coded into appropriate variable and entered in to a Microsoft Office Excel spreadsheet. The data

was checked for errors entry, coded and then imported to SPSS for descriptive and further analyses. All statistical analyses were performed using SPSS version 26 software. Binary logistic regression was used to study associated risk factors.

Results

Serological Result with Associated Risk Factors

Indirect ELISA test was used to test serum samples from chickens in Gondar city and three districts in northwest Ethiopia for previous exposure to infectious bronchitis virus. 354 of the 384 blood sera tested for infectious bronchitis virus antibodies were positive. The prevalence of IB was found to be 92.19% (89%-94.6% with 95 CI %). Table 1 shows IB seroprevalence in Gondar city and three districts; with no significant difference between them ($p > 0.05$). The prevalence of the disease was found to be 96.66% in west Dembya, 90.82% in East Dembya, and 85.39% in Gondar Zuria. A significantly higher prevalence was discovered in young chickens (95.83%) compared to adults (87.5%) with COR 3.286 (95 % CI 1.463-7.378) ($p < 0.05$). The prevalence of IB was also higher in exotic breeds (94.57%) with COR 3.903(95 % CI 1.798-8.471) than local breeds (81.69 %) with a significant difference ($P < 0.05$). The prevalence in females (92.7%) was higher than males (90.98 %) with COR 1.267(95 % CI 0.583-2.753) but the difference was not significant ($p > 0.05$). Though the difference was not statistically significant ($p > 0.05$) the prevalence in intensive farms was higher (93.39%) than the prevalence in extensive farms (90.69%) with COR 1.451(95 % CI.687-3.063). Higher Seroprevalence (93.75%) was observed in dual purpose chickens followed by layers with a prevalence of (92.7%) and broilers (90.98%) but the difference was not statistically significant ($p > 0.05$) (Table 2).

	No. of Chickens Examined	No. of Positive	Prevalence	Univariable COR (95% CI)	p value
Origin					
Gondar City	108	103	95.37%	Reference	
West Dembya	89	86	96.63%	1.392(0.323-5.990)	0.657
East Dembya	98	89	90.81%	0.480(.155-1.485)	0.203
Gondar Zuria	89	76	85.39%	0.284(.097-.830)	0.21
Total	384	354	92.19% (89%-94.7%)		

COR: crude odds ratio; 95% CI: 95% confidence interval. P values < 0.05 were statistically significant.

Table 1: Univariable Logistic Regression Analysis Result in Relation with Study Area.

Variables	No. of Chickens Examined	No. of Positive COR (95% CI)	Prevalence	Univariable	p value	Multivariable AOR (95% CI)	p value
Age							
Young	216	207	93.83%	3.286(1.463-7.378)	0.004	4.122(1.451-11.715)	0.008
Adult	168	147	87.50%	Reference			
Breed							
Local	71	58	81.69%	Reference			
Exotic	313	296	94.57%	3.903(1.798-8.471)	0.001	3.980(1.147-13.809)	0.03
Sex							
Male	122	111	90.98%	Reference			
Female	262	243	92.70%	1.267(.583-2.753)	0.549	1.665(.368-7.528)	0.508
Production System							
Intensive	212	198	93.39%	1.451(.687-3.063)	0.329	1.460(.518-4.117)	0.475
Extensive	172	156	90.69%	Reference			
Production Purpose							
Broiler	122	111	90.98%	Reference			
Layer	166	153	92.17%	1.166(.504-2.7)	0.719	1.366(.409-4.563)	0.612
Dual	96	90	93.75%	1.486 (.529-4.176)	0.452	1.665(.368-7.528)	0.508

COR: crude odds ratio; AOR: adjusted odds ratio; 95% CI: 95% confidence interval. P values <0.05 were statistically significant.

Table 2: Univariable and Multivariable Logistic Regression Analysis Result in Relation with Associated Risk Factors.

RT-PCR Result

Samples that were injected in to SPF eggs were collected after 96 hours and passaged three times and were taken in to molecular biology laboratory for PCR analysis. The test was performed following the standard protocol. Among the

26 pooled samples tested 3 (11.54%) of them were positive during RT-PCR examination. Amplification of an expected DNA band (466 bp) from positive control as well as IBV positive swab samples indicated that the RT-PCR reaction has been performed correctly (Figure 2).

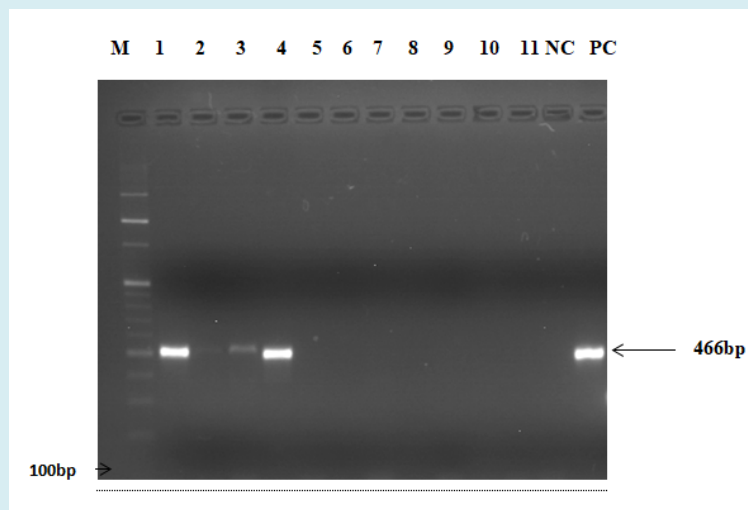


Figure 2: The Detection of IBV by RT-PCR. Key: Lane M: molecular ladder started at 100bp, Lane 1, 3, 4 positive samples around 466bp, Lane 2 and 5-11 Negative Samples, Lane NC – Negative Control, Lane PC –Positive Control around 466bp.

Discussion

A cross-sectional study was undertaken on serum samples from 384 chickens to determine seroprevalence of infectious bronchitis and 26 pooled tracheal swab samples for molecular detection of the virus in central Gondar zone. All of the chickens in this investigation were not vaccinated against infectious bronchitis virus (IBV). Based on the current investigation 92.19% of the selected chickens in the study area were positive for infectious bronchitis. Accordingly this study discovered the highest prevalence of infectious bronchitis as compared to the study carried out by Birhan, et al. [9] in northern Ethiopia who reported a seroprevalence of (24.6%). The result of this study was consistent with those of previous studies by Hutton, et al. [12], who discovered a prevalence of 94.5 % in Debrezeit, Ethiopia and Shiferaw, et al. [13] who reported a seroprevalence of 97.46 % in Bishoftu, Ethiopia. The current study was comparable to those from Nigeria by Owoade, et al. [14], Emikpe, et al. [15] and Mungadi, et al. [16], as well as those from Pakistan by Benazir, et al. [17] who found seroprevalence of 84%, 82.7%, 89%, and 84.40%, respectively.

The finding of this study was higher than the study conducted by Tesfaye, et al. [18] and Yonas, et al. [19] who found 70.6 % and 64.7 % seroprevalence of IBV in Sebeta, Hawassa, and Ada'a Districts of Ethiopia, respectively. The result of the current study was also higher than the studies by Barberis, et al. [20] from Algeria, Thekiso, et al. [21] from South Africa and Kouakou, et al. [22] from Ivory Coast who reported a prevalence of 78.25%, 43% and 72% respectively. The finding of this study was also higher than the study by Das, et al. [23] and Bhuiyan, et al. [24] from Bangladesh who found a Prevalence of 79.38% and 59.30% respectively. Variations in agro climatic condition and management practices could explain the differences in seroprevalence. Furthermore, the difference in seroprevalence could be linked to a rise in IBV activity among chickens in the research area [25].

The prevalence varied significantly among chickens of different ages ($p < 0.05$). Young chickens (95.83 %) had the higher prevalence than adults (87.5 %). This result was in line with that of Mungadi, et al. [16], who observed that there was a significant difference between the prevalence in growing and adult chickens. This result was also in line with the study by Cavanagh, et al. [3] who claimed that while all age groups of chicks are susceptible to IBV; young chicks are more vulnerable than older ones. Additionally, as chickens get older, their resistance to diseases is stronger as well. The prevalence of the disease was higher in exotic breeds (94.57%) than the prevalence of in local breed (81.69 %). This might be connected to exotic breeds limited resistance to disease and other environmental stresses [26].

An investigation of the prevalence of IB in chicken farms during the study found that intensive farming had higher seroprevalence (93.39%) than its extensive equivalents (90.69%), although the difference in susceptibility was statistically insignificant ($p > 0.005$). The higher prevalence rate recorded in intensive farms in this study was consistent with reports from commercial poultry farms in Nigeria (91.3%) by Oyejide, et al. [27] in Jordan (92.9%) by Rousson, et al. [28] and in Ethiopia (94.5%) by Hutton, et al. [12]. The finding of this study was also consistent with the study by Shettima, et al. [29] who indicated an increase seroprevalence in intensive farming. This might be due to the various management protocol types. Given that contact between regions that produce poultry has been linked to the development and maintenance of infectious diseases, this may also be related to the existence of farms in nearby areas [30] and [31].

The current study found higher seroprevalence in layers (93.75%) and dual-purpose chickens (92.17%) as compared with broilers (90.98 %). This finding is in agreement with the study by Shettima, et al. [29] who explains that there was a significant difference in the prevalence among layers and broilers. This could be explained by layer and dual-purpose birds spending more time on the farm than other birds, which leads to their re-infection if there is no effective way to manage it [32]. This study was also in line with the explanation provided by Javed, et al. in [33] who found that the longer the chickens were exposed to the virus, the higher the seroprevalence of IB became.

Though the difference was not statistically significant a higher seroprevalence was obtained in females (92.7%) than in males (90.98 %). This outcome is consistent with the study of local chickens in live bird markets in Sokoto State, Nigeria, who found a higher prevalence in females than in males with insignificant difference. Due to differences in the activity of humoral- and cell-mediated immune responses between the sexes, this may be connected to the presence of a less effective immunological response in males than in females [16].

Isolation of the virus in embryonated eggs, followed by immunological identification of the isolates, is frequently used to make the diagnosis of infectious bronchitis (IB). This is a tedious process and necessitates the employment of particular polyclonal or monoclonal antibodies [34]. Additionally, some isolates can be combinations of various IBV types, which complicate the interpretation of serotyping results. There have been prior descriptions of reverse transcription PCR employing IBV, RNA isolated from allantoic fluid, and tracheal swabs. These methods have been proven to be quite effective for both IBV detection and IBV type identification [35].

As far as molecular detection of the virus is concerned this is the only study we are aware of that describes IBV molecular detection in the study area. The infection is believed to have started in the field since the farms did not use IBV vaccine, as was previously described in this article. This study was in agreement with the study by Hutton, et al. [12] in other research regions of Ethiopia, who reported molecular detection of IBV. The prevalence in this study was higher (11.54%) than the study by Tegegne, et al. [8] who reported a prevalence of 6% in Jimma zone, Ethiopia. This could be due to the difference in implementing biosecurity measures.

Conclusion and Recommendations

The result of the current study shows higher prevalence of the disease (92.19%) and RT-PCR result provides confirmation of the presence of IBV with a prevalence of (11.54%). Accordingly, this is the first molecular evidence that the study area harbors an infectious bronchitis virus.

Based on the above conclusion the following recommendations has been forwarded

- Major method of prevention for the disease like vaccination against the disease should be practiced.
- When new IBV strains appear in a particular area, an IB vaccination program is unsuccessful. In order to select an appropriate virus strain for vaccination, it has been advised to regularly monitor the IBV strains that are currently present in a certain geographic area.
- To lessen the impact of the disease, additional biosecurity measures must be put in place.
- In order to handle an efficient measurement system from the study area in specific and at a country level in general as a future study, sequencing and genotyping of IBV are recommended.

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Authors' Contributions

Conceptualization, methodology, formal analysis, investigation, resources, writing-original draft preparation, T.M. and M.B., O.O., data collection, T.M., G.E. and D.W. performing laboratory work T.M. G.D., K.B O.A and A.L.; Writing-review and editing, T.M. and M.B., O.O. All authors have read and agreed to the published version of the

manuscript.

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Availability of Data and Materials

This article contains all of the data that was created or analyzed throughout the investigation.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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