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ORIGINAL RESEARCH

Serological Investigation of Newcastle Disease in Selected Districts of Buno Bedelle Zone, Ethiopia

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Purpose: Newcastle disease causes considerable loss in the poultry industry worldwide and its outbreaks are regularly reported from all continents of the world, including Ethiopia. The investigation was conducted from October 2020 to June 2021 to determine the prevalence of Newcastle disease (ND) in districts selected from Buno Bedele zone, Western Oromia.

Methods: The study design used was a cross-sectional type. For this study, a total of 480 serum samples were collected as per sample collection guideline from randomly selected chickens that were apparently healthy and had no history of vaccination against Newcastle disease. Indirect ELISA was performed and all data were analyzed using SPSS statistical software.

Results: From serological investigation, overall seroprevalence of Newcastle disease was 30%. Seroprevalence of Newcastle disease was 34.94%, 22.22%, and 31.76% in Didessa, Chora, and Gachi districts, respectively. Among computed risk factors, breed showed statistically significant difference and the odds of infection were lower in adult than in young chickens. Similarly, the odds of infection with Newcastle disease virus were significantly higher in crossbred than in locally bred backyard chickens.

Conclusion: This study revealed Newcastle disease was prevalent in the study areas; therefore, regular investigation of Newcastle disease should be conducted along with detailed studies on molecular characterization of circulating field strains in the area.

Keywords: Buno Bedele, Ethiopia, indirect ELISA, Newcastle disease

Introduction

Poultry in Ethiopia serves as critical contributor to food and nutrition security, and is a source of cash earning for a large part of the population.[1,2] As in many developing countries, chickens are extensively kept in Ethiopia,[3] with the overall population predicted to be approximately 60 million of which 90.8%, 4.4% and, 4.8% had been suggested to be indigenous, exotic and, hybrid, respectively.[4]

Poultry diseases are responsible for several adverse financial and social impact including mortality and morbidity of chickens, excessive medication costs, loss in manufacturing and marketing that could pose a risk to public health through zoonoses.[5] Newcastle disease is a highly contagious viral disease affecting wild and domestic avian species.[6,7] It is caused by virulent viruses from the genus Avulavirus and species avian avulavirus 1, usually called Newcastle disease virus (NDV) and abbreviated as avian paramyxovirus 1 (APMV1).[8,9]

The Newcastle disease virus (NDV) strains are categorized as velogenic, mesogenic, and lentogenic primarily based on their pathotypes and virulence.[10,11] ND virulent strains are related to intense financial losses because of excessive morbidity

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and mortality, drop in egg-laying, and lesions in the upper respiratory and digestive tracts.[12] It is a serious epizootic chicken disease in the low-earning food-deficit countries. It happens every year and kills on average 70% to 80% of the unvaccinated free-range indigenous chickens.[13,14] It is endemic in Ethiopia with common outbreaks in the commercial, outdoor, and village chicken farms.[15,16]

Clinical diagnosis based on history, signs, and lesions further to hemagglutination and hemagglutination inhibition test, virus neutralization test, enzyme-linked immunosorbent test, plaque neutralization test and reverse transcription-polymerase chain reaction (RT-PCR) may be used for confirmation of the ND virus.[13]

Previous sero-epidemiological investigations in different parts of Ethiopia[17,18] indicated the endemicity of ND in village chickens. Newcastle disease is one of the problems in village chickens in most parts of Ethiopia.[19,20] In Ethiopia, NDV has been stated to be widespread because of fast expansion of the poultry industry, excessive stocking densities, and insufficient biosecurity measures which create situations conducive for the spread and maintenance of the endemicity of the disease.[21]

The disease is extensively distributed in the world and several countries such as Asia, Africa, Central America, and South America have endemic or common outbreaks due to virulent Newcastle Disease Virus (NDV) and there are sporadic outbreaks of the virus internationally.[22] In worldwide spread, ND with low virulent strains is enzootic in Asia, Africa, Central America, and a part of South America, representing a permanent hazard to the poultry industry. The availability of serological test which is tailored to the conditions in these countries would facilitate diagnosis and accurate monitoring of vaccination programs.[23]

Despite the rampant outbreaks and the truth that ND represents the most intense chicken disease, epidemiological study regarding the disease specifically in BunoBedele zone are scarce. Continuous surveillance of the disease is important to take measures which includes appropriate control practices including vaccination of the susceptible chickens.

In line with this, the aim of the study is to determine the seroprevalence of Newcastle disease and its associated risk factors in non-vaccinated village chickens of the three selected districts of Buno bedele zone.

Materials and Methods

Study Area

The investigation was carried out in three districts (Didessa, Chora, and Gechi) of Buno Bedele zone.

Didessa district is one of the districts of Buno Bedele zone, Oromia National Regional State. Administratively the district is divided into 31 rural kebeles and one town. It is located at a distance of 60 Km from the zonal town and 420 Km from the capital Addis Ababa. Geographically, the Didessa district extends between 7° 59′ 14″ to 8° 14′ 48″ North and 36°16′56” up to 36° 49′ 21″ East.[24]

Chora is one of the 21 districts of Buno Bedele zone in Oromia region. It is 515 km away from Addis Ababa on the main road to Bedele to Metu. This town has a longitude and latitude of 8°27′N 36°21′E and an elevation between 2012-2162 meters (6601-7093 ft) above sea level.[25]

Gechi Woreda is found in Buno Bedele zone of Oromia Region, southwestern Ethiopia. It is located at a longitude and range of 8º27ˈN36º21ˈE and 8.450ºN36.350ºE respectively at approximately 462 km southwest of Addis Ababa. It is bordered on the south by Didessa, on the East by the Jimma, on the north by Bedele of Benishangul Gumuz Region, on the northeast the Didessa River which separates it from the East Wollega zone, on the southeast by Gechi.[26]

Study Animals

Domestic chickens were sampled with respect to factors including district, sex and age. The age was determined based on history from the owners (27), as young, adult, and old.

Study Design

The study was cross-sectional in its design. The purpose of the study was explained to the selected owners before sample collection. Didessa, Gechi, and Chora districts of Bedele zone were the involved study areas. Figure 1 indicates number of collected serum samples in each district of the study area.

Sample Size Determinations

A total of 480 serum samples were collected from randomly selected chickens. Initially three districts were selected from the zone. Three peasant associations were selected from each district using random sampling method. The sample size was decided using the Thrusfield formula[28] considering prevalence of ND in the study areas was 50%, with a 95% confidence interval.

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

N= the needed sample size, P exp= expected prevalence and d = desired absolute precision, N=384. To increase

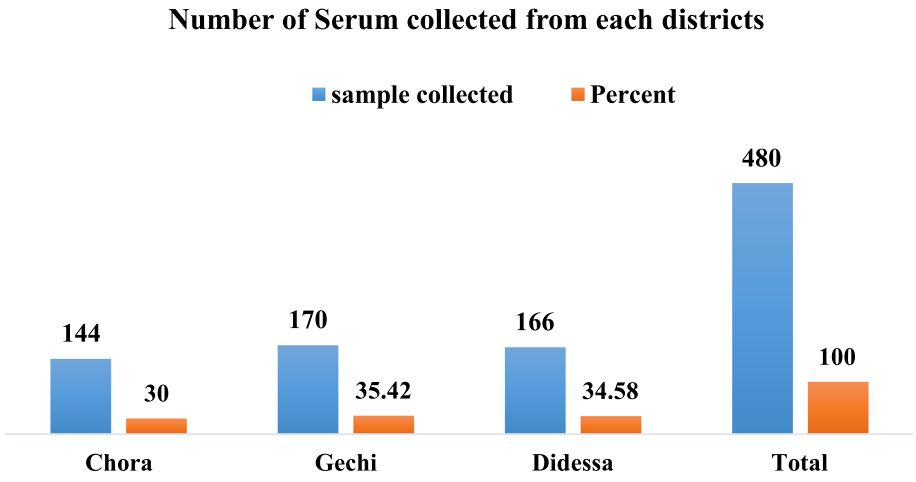

Figure 1 Number of serum samples collected in each district of the study area.

precision by 25%, additional 96 backyard chickens were added. Hence 480 chickens were used for the study.

Sample Collection and Laboratory Procedures

After disinfecting the site, 2–3 mL of blood was collected from the brachial vein of chickens using a 5mL syringe and a 23 gauge. Then, collected blood was labeled and allowed to clot overnight under normal atmospheric conditions. So, clear serum was harvested in labeled 2mL cryovial tubes and stored at −20°C until indirect ELISA was carried out.[23]

An indirect ELISA technique was carried out through the use of IDvet Innovative Diagnostics kits (Grabels, France): ID Screen® NDV Indirect. All the reagents were adapted to room temperature (21 ± 5°C) before use and homogenized afterward by inversion. The sera were diluted to 1/500th and then loaded to ELISA plates to start an immunosorbent reaction as per the manufacturer's manual. ELx800 spectrophotometer (BioTek™, USA) equipped with 450 nm filter where the measured optical density was transformed into titrated antibody read ELISA plates. The averages of the titers and the coefficient of variation (CV) were automatically calculated by the band and by series of samples, with the software provided by the laboratory (Gen 5 1.11).

Data Management and Analysis

The data generated from the study were arranged, coded, and entered into an Excel spreadsheet (Microsoft® excel 2007). SPSS version 20 statistical software was used for analysis including Pearson's chi-squared test and logistic regression. Logistic regression analysis was done to determine association between the seroprevalence and associated risk factors. P value less than 0.05 indicated statistically significant differences.

Results

From the total 480 chickens' sera examined in three selected districts of Buno Bedele zone, 144 (30%) of them were identified as positive for antibodies against NDV. The highest ND seroprevalence (34.94%) was recorded in Didessa, followed by Gachi (31.76%), and Chora (22.22%). Table 1 indicates seroprevalance of Newcastle disease in the three districts of Buno Bedele zone.

Among the sex groups, a higher prevalence (31.6%) was observed in female animals. Breed-wise, crossbreeds showed the highest level of ND seropositivity (36.8%). Seroprevalence of Newcastle disease in association with other risk factors like breed, age, and sex, are shown in Table 2. In the cross- and local breeds of chickens, the prevalence was (34.37%) and (9.74%) respectively. Only breed showed statistically significant differences among risk factors computed in Buno Bedele zone. Association between risk factors and ND seropositivity was indicated in Table 2.

The logistic regression analysis identified that breed was statistically significant (Table 3). Cross-bred chickens were more likely to be infected by ND than local breeds (OR = 0.251, 95% CI: 0.145–0.433). The risk of ND occurrence was also increased 0.607 times (OR = 0.607, 95% CI: 0.33–01.117) in female animals than in males. The results also showed that the odds of infection were higher in old chickens than in young and adult chickens. Table 3 indicates logistic regression analysis of ND seropositivity with various risk factors.

Table 1 Seroprevalance of Newcastle Disease in Districts of Buno Bedele Zone

Zone	District	PA	Total No of Samples	No of Positive Samples	No of Negative Samples	Prevalence %
Buno Bedele	Didessa	Yembero	56	23	33	41.07%
		Sineso	60	22	38	36.67%
		Garado	50	13	37	26%
		Total	**166**	**58**	**108**	**34.94%**
	Chora	Schickengela	50	10	40	20%
		Aba Bora	50	12	38	24%
		Hawa Yember	44	10	34	22.72%
		Total	**144**	**32**	**112**	**22.22%**
	Gachi	Seko	67	16	51	23.88%
		Gole Maya	50	17	33	34%
		Gachi 01 Kebele	53	21	32	39.62%
		Total	**170**	**54**	**116**	**31.76%**
		480		**144**	**336**	**30%**

Note: $X^{2=}491$, P-value=0.005.

Table 2 Association Between Risk Factors and ND Seropositivity

Risk Factors		No of Chickens Tested	Positive	% Positive	X^2	P-value
Sex	Male Female	78 402	17 127	21.8% 31.6%	2.5375	0.1112
Breed	Local Cross	138 342	18 126	13.0% 36.8%	25.397	4.666e-07
Age	Young Adult Old	80 347 53	25 99 20	31.2% 28.5% 37.7%	1.9268	0.3816

Table 3 Logistic Regression Analysis of ND Seropositivity with Various Risk Factors

Variables	Level of Variables	OR	CI	P-value
Sex	Female vs Male	0.607	0.330–1.117	0.1112
Breed	Cross vs local	0.251	0.145–0.433	4.666e-07
Age	Young, Adult vs Old	0.939	0.740–1.192	0.3816

Abbreviations: OR, odds ratio; CI, confidence interval.

Generally, the overall seroprevalence of ND showed that females [(31.59%) 127/402] were more affected than males (21.79%) 17/78), while breed-wise, cross-bre d [(36.84%) 126/342] chickens were more affected than local breeds [(13.04%) 18/138].

Discussion

The present study revealed circulating NDV in backyard chickens in different districts of Buno Bedele zone of Western Oromia, Ethiopia using indirect ELISA. The overall prevalence of ND in backyard chickens in this study was 30%.

The overall seroprevalence in this study was in close agreement with the findings of earlier studies in central Ethiopia at Debre Berhan which showed a prevalence of 28.57%[17] and 23.6% in the Delta State of Nigeria.[29] However, the present findings were considerably lower than in the previous reports by Tadesse et al[17] in which a seroprevalence of 32.2% was reported in central Ethiopia. On the other hand, the result of the present study was higher than the findings of other studies in Eastern Shewa Kersa kondaltity district and rift valley areas of Ethiopia where seroprevalence of 5.9%, 5.6%, and 11.61% was recorded respectively.[6,30,31] So, the present study revealed that ND is endemic in the studied area and all PAs in the districts showed evidence of Newcastle disease in the laboratory tested sera. The variable seroprevalence rates ranging from 20% to 41.07% in this study agree with the findings of various researchers in Ethiopia. This finding was consistent with the previous study reports in different parts of Ethiopia, 12.9 to 47.6% in Southern and Rift valleys[18] and 32.22% in the central part of Ethiopia.[17] The prevalence varied across different study areas. From Buno Bedele zone selected districts, the highest prevalence was recorded at Didessa (34.94%) and the lowest prevalence was recorded at Chora (22.22%) district. In this study, the prevalence of ND antibodies for sex was determined and seroprevalence in female chickens was relatively higher (31.6%) than in males (21.8%) in Buno Bedele. The difference was not statistically significant (x^2= 2.5375; P value=0.1112). This result was similar to previous study findings reported in Ethiopia.[18,27]

From the study findings, female chickens were highly infected by NDV compared to male chickens. According to[17,32–35] in Ethiopia, female chickens were more vulnerable to NDV compared to male chickens, which is consistent with this study. This could be because female chickens were mostly retained for long-term production purposes compared to the males which were mostly used for non-productive purposes (food, income, and socio-cultural or religious rites). The prevalence of NDV in different breeds was varied.

More cross-bred chickens [126 (36.84%)] were positive than locally-bred [18 (13.04%)] in Buno Bedele. There was a statistically significant difference (x^2= 25.397; P-value=4.466e07). Regarding breed of chicken, it was higher (36.84%) in cross-breeds than in local breeds (13.04%). The variation could be multifactorial like management, season, unequal proportions of breeds sampled during sampling.[36]

Seroprevalence was also assessed by age and the difference in the seroprevalence between young and adult chickens was statistically insignificant (p>0.05), which does not agree with the finding of Vui et al,[37] which stated that young chickens had a significantly lower NDV antibody titer than adults. Overall, in Buno Bedele, seroprevalence was high in old age chickens and this can be hypothesized to be due to more frequent exposure of older birds to field virus, which means they might have survived the disease at an earlier age.[27]

Female chickens were more likely to be infected with Newcastle disease virus (OR=0.607; 95% CI: 0.330–1.117) than male chickens. Additionally, cross-bred chickens were more likely to be infected with Newcastle disease virus (OR=0.251; 95% CI: 0.145–0.433) than locally-bred chickens. The overall variation of the findings might be due to variation in management system that may serve as a stress factor and favor infection. Poor sanitary conditions, continuous exposure of chickens to range conditions and wild birds, nutritional deficiencies, absence of vaccination in traditionally managed chickens, and contact of chickens of one village with those in other villages may facilitate the spread of ND.[38] Generally, findings of this research indicated ND is a prevalent disease in the study area. Hence, attention should be paid to control ND prior to outbreaks.

Conclusion and Recommendations

Newcastle disease was successfully investigated in this study. Findings of seroprevalence among backyard chickens indicated that ND is a serious health problem. Overall seroprevalence of ND in this study was 30%. The disease affected all age groups of chickens regardless of the differences in breed and sex. In conclusion, chickens are endemically infected with NDV in the study areas. Hence, detailed investigation coupled with molecular analysis should be conducted to assess the status of NDV infection and give insight for further investigations.

Data Sharing Statement

It is incorporated in the main text or article.

Ethical Consideration

The research was carried out with high regard for animal welfare and it was approved by Wollega University, Department of Veterinary Clinical Science.

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Disclosure

The authors report no conflicts of interest for this work.

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