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PREVALENCE OF NEWCASTLE DISEASE USING THE HAEMAGGLUTINATION INHIBITION (HI) TEST, AND THE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) AMONG LOCAL EXTENSIVELY REARED CHICKEN IN SIERRA LEONE

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ABSTRACT

Newcastle disease (ND) is a viral infection with a global distribution that poses significant economic implications and a serious threat to nutritional security. This highly contagious disease is often marked by high morbidity and mortality rates, creating a substantial public health risk. Consequently, identifying effective alternative diagnostic methods, such as the Enzyme-Linked Immunosorbent Assay (ELISA), is crucial for the early detection and control of ND. The aim of this study was to estimate the seropositivity of Newcastle disease virus (NDV) by comparing the sensitivity of Hemagglutination Inhibition (HI) and ELISA tests among 800 free-range chickens across four regions in Sierra Leone. Our findings revealed a higher seropositivity rate using the ELISA test (13.9%) compared to the HI test (9.8%). The eastern region was found to be the most affected, with seropositivity rates of 43.6% for ELISA and 42.3% for HI. Among the risk factors assessed, chickens aged one year and older (ELISA = 11.2%, HI = 11.7%) and male chickens (ELISA = 19.1%, HI = 13.0%) were identified as the most susceptible groups. This study concludes that the ELISA technique demonstrates superior sensitivity for the serological detection of ND virus, while also being less labor-intensive and time-consuming than traditional methods. The high seropositivity rates indicate widespread exposure to NDV in the country. To effectively control the disease, we recommend enhancing community awareness, improving husbandry practices, and

implementing regular vaccination programs. These measures are essential to mitigate the impact of Newcastle disease on poultry health and local food security.

Keywords: ELISA and HI test, Local chickens, Newcastle disease, Risk factors, Seropositivity, Sierra Leone.

INTRODUCTION

Livestock diseases have a significant impact in many developing countries where most of the people live in rural areas and depend on agriculture for their livelihoods. Livestock production provides a good source of food through meat milk and eggs [1]. Livestock may also be a form of savings, which can be converted into cash for exigency purposes. In addition to the benefits accrue to individual livestock owners; both small holders and intensive livestock industries may make a large contribution to national economy. In many developing countries, outbreak of major diseases occurs frequently, and are poorly controlled resulting in large numbers of death. The constraints poised by disease are particularly important when birds are housed under different conditions such as the intensive or extensive system of management. Many of the diseases and parasites that cause problems are common to the tropics, sub tropics, and temperate zones. Conversely new diseases have also emerged in countries where they have never been and also some of these diseases have also been found to infect human beings as well. Therefore, development partners and government initiatives should be directed towards solving the problems of animal diseases.

Newcastle disease is a viral disease of birds with a wide range of clinical signs from mild to severe. The highly virulent form of Newcastle disease is one of the most important poultry diseases Worldwide. Among the different species of birds (at least 200) being infected by the virus and depending of the host and viral strain, chickens are the most susceptible birds [2]. ND is a highly contagious disease with morbidity and mortality rates up to 100% [3-4] in a naïve population. Outbreaks of virulent Newcastle disease have a tremendous impact on backyard chickens in developing countries, where these birds are a significant source of protein and household income and this disease is endemic. In developing countries, where the more virulent forms of the virus have not been eradicated, trade embargoes and restrictions cause significant economic losses during outbreaks.

Like many other developing countries, chickens are the most abundant and widely reared livestock in Sierra Leone. According to a survey conducted by the Food and Agriculture Organization of the United Nations, the poultry population in the country is approximately 14,721,718, accounting for about 83.5% of the national livestock population [5]. Notably, around 80-85% of the chicken population is owned and raised by women, reflecting their crucial economic and social roles.

Poultry production primarily serves two purposes: providing meat and eggs for household consumption and supporting breeding activities. Income generation and cultural practices also contribute to the well-

being of the rural population. However, poultry, particularly chickens, are often raised under low management conditions. Approximately two-thirds of poultry farmers do not provide treatment, vaccination, or planned supplementary feeding for their flocks. Instead, chickens primarily scavenge for food, relying on insects, leftovers, and wild vegetation.

Chickens are highly prolific and exhibit resistance to many prevalent diseases and parasites, enabling them to thrive in harsh environmental conditions. The chicken population tends to vary seasonally, with the highest numbers typically observed in the early dry season, which coincides with the harvest and process period. This population consists of various breeds across different age groups and sex categories, all managed under a common free-range system.

The poultry sector in Sierra Leone faces significant challenges, including inadequate veterinary services, suboptimal husbandry practices, a lack of support programs, rampant inbreeding, and high morbidity and mortality rates due to diseases and predation [6]. Major diseases affecting the sector include Newcastle disease, fowl pox, and coccidiosis, which lead to substantial losses through mortality and decreased productivity. Addressing these challenges is critical for improving the resilience and productivity of the poultry sector in Sierra Leone.

In Sierra Leone, Newcastle disease (ND) is the most devastating viral disease affecting poultry, characterized by high mortality rates and significant economic consequences. The disease is endemic in the country, with outbreaks typically occurring during the early dry seasons (December to February and April to June), along with sporadic incidents in other months.

During the onset and conclusion of the dry season, Sierra Leone sees an influx of migratory birds, which often show no signs of illness, compounding the risk of disease transmission in conjunction with changing weather patterns and climate variability. Recent national surveillance reports have linked the emergence of Newcastle disease outbreaks to various ecological factors. Communities in coastal regions, highland areas, peri-urban settings, and cooler climates tend to report higher incidences of the disease.

Each year, farmers suffer substantial losses due to ineffective control measures, poor biosecurity practices, inadequate husbandry techniques, and limited local knowledge about the disease. These challenges highlight the urgent need for improved awareness, education, and intervention strategies to mitigate the impact of Newcastle disease on the poultry sector and enhance the livelihoods of affected farmers.

Sierra Leone relies heavily on agriculture, particularly livestock, as a primary source of animal protein. However, the economic implications of Newcastle disease (ND) in the country remain largely unquantified and poorly understood due to a lack of empirical evidence. The outbreaks of ND have significant consequences, both direct and indirect. Direct effects include high mortality rates in affected flocks, abandonment of eggs by laying hens, and poor body condition in surviving birds. Indirect impacts encompass treatment costs, loss of income resulting from decreased production, reduced household

protein availability, and restrictions on live bird trade. Beyond the financial losses incurred by farmers, ND threatens local chicken genetic resources, leading to the extinction of less common breeds and adversely affecting the livelihoods of many impoverished Sierra Leoneans. Additionally, ND poses a public health concern for individuals in close contact with poultry, including poultry workers and animal health professionals. While ND typically causes mild, self-limiting symptoms in humans upon exposure [7], the potential for transmission underscores the importance of effective management and control measures to safeguard both animal and human health.

However, Newcastle disease is regarded as a neglected issue in Sierra Leone despite being a notifiable disease. This neglect can be attributed to its limited research on its economic impact, small flock sizes, unnoticeable public health threat, and a weak veterinary service. The lack of attention to the poultry industry from both government and development partners has facilitated the rapid spread of the disease, leading to the exposure and death of hundreds of chickens annually.

In the absence of effective vaccination programs, farmers have implemented conventional strategies to mitigate the spread of ND at the farm level. These control measures primarily focus on management and sanitary practices, such as culling and isolating infected birds, careful disposal of dead animals, maintaining good hygiene, and using traditional remedies to treat symptomatic birds. For decades, these methods have proven effective in reducing the impact of Newcastle disease in affected flocks.

Newcastle disease, also referred to as avian paramyxovirus type 1 [8], is a highly infectious viral disease that impacts various species of domestic and wild birds globally. Caused by the Newcastle disease virus (NDV), it can result in substantial economic losses for the poultry industry due to high mortality rates among infected flocks.

In Sierra Leone, where the veterinary delivery services is faced with many challenges outbreak of livestock diseases could have devastating effects. Typical of many armed conflicts in Africa, livestock particularly indigenous chickens suffer equal atrocities like humans. They serve as food for armed groups. During the ten years (1991-2002) civil strife in Sierra Leone, majority of livestock and livestock facilities were decimated. Immediately after the cessation of hostilities, a mass indiscriminate restocking exercise was undertaken to assist the rural populace resettle in their farming communities. Unfortunately, this restocking exercise ignored very important issues such as procurement of healthy and reproductive animals, quarantine of livestock to ensure disease-specific free livestock were imported, and timely vaccination before and after restocking. This resulted in the proliferation of animal diseases such Pests des Petit Ruminants (PPR) and Newcastle disease (ND). These diseases are seriously causing serious economic problems not only to farmers but to the country as a whole.

Very few studies have been conducted in this area using only ELISA diagnostic method. The aim of this research is to understand the extent of Newcastle disease on chicken populations in the twelve districts of Sierra Leone using two different serological methods to determine the extent of the viral disease. The

primary goal is to gather data that can help in developing effective control and prevention strategies to mitigate the spread of Newcastle disease among chicken in Sierra Leone. The specific objectives of the study are:

1. To determine the prevalence rate of Newcastle disease among chicken in different regions of Sierra Leone.
2. To compare the two serological tests (HI and ELISA) in diagnosing Newcastle disease
3. To propose recommendations for improving disease surveillance, prevention, and control strategies to reduce the burden of Newcastle Disease in Sierra Leone.

The following research questions guide this diagnostic study:

1. What is the prevalence rate of Newcastle Disease among local chickens in Sierra Leone?
2. What is the difference between HI and ELISA in diagnosing Newcastle disease in chicken?
3. Which region is most susceptible to Newcastle disease infection?
4. What are the intrinsic risk factors associate with the susceptibility of NDV?

METHODOLOGY

Description of study area

The study was conducted across various regions and districts of Sierra Leone, where extensive local poultry farming is predominantly practiced at the small-scale traditional level. In all provinces, indigenous poultry farming serves as a vital source of household income and is deeply woven into the cultural and social fabric of rural communities. It bolsters local economies through direct sales and provides subsistence for many families. The trade of live birds and their products significantly contributes to the economic stability of these regions.

Extensive poultry farming systems are typically located in rural areas, where chickens are raised with minimal confinement and often receive limited veterinary care. These conditions create an ideal environment for assessing the prevalence of Newcastle disease (ND), given the high likelihood of exposure to the virus and the variability in vaccination practices. By focusing on these settings, the study aims to provide valuable insights into the epidemiology of ND and inform strategies for improving poultry health and biosecurity in the region.

The extensive poultry rearing systems in these areas consist of various flocks of chickens that roam freely within designated premises. These systems are typically characterized by low-density housing, limited access to veterinary services, and a high likelihood of contact with wild birds and other potential sources of infectious diseases, such as Newcastle disease virus (NDV). Such conditions can facilitate the spread of ND and undermine the effectiveness of vaccination programs.

The local poultry population primarily comprises indigenous or local breeds, which tend to be more resilient to environmental stresses but may exhibit varying levels of immunity to different pathogens. According to a survey conducted by the Food and Agriculture Organization of the United Nations in 2016,

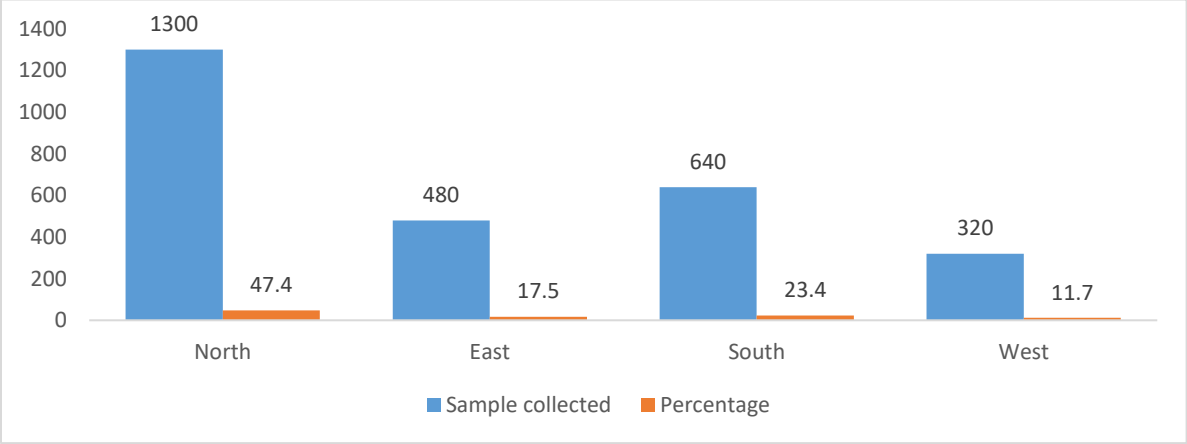
the poultry population in Sierra Leone is approximately 14,721,718 (Agyemang et al. 2017). These chickens are typically raised for both egg production and meat, with farming practices ranging from traditional to semi-commercial methods. This diversity in rearing practices reflects the adaptation of local farmers to their environmental and economic conditions, but it also poses challenges for disease management and biosecurity.

The study area is characterized by a diverse ecosystem, including extensive grasslands, mixed vegetation, tropical forests, mangroves, and coastal wetlands. The climate is classified as monsoon humid tropical, featuring distinct wet and dry seasons, with a prolonged rainy season lasting approximately 7 to 8 months. Annual rainfall can exceed 3,000 mm in certain regions, peaking in August. The average annual temperature in the area is 26.7°C, contributing to the region's rich biodiversity and influencing the local agricultural practices.

Study design and sampling procedure

A cross-sectional study was conducted to estimate the seroprevalence of Newcastle disease (ND) at the household level in affected communities, utilizing both hemagglutination inhibition (HI) and ELISA assays. The study was purposefully designed to collect blood samples from targeted local chicken populations across all districts in Sierra Leone over a one-year period, from January to December 2018. Sixty towns and villages reporting suspected ND outbreaks were identified and included in the study, as detailed below. Information regarding the suspected incidence of ND outbreaks at the time of the study was gathered with the assistance of animal health workers, livestock officials, and farmers residing in the affected communities.

Chickens aged four months and older, reared in a free-range system for at least one year, were sampled regardless of their sex or breed. The sample size for each region or district was determined based on the number of communities that reported outbreaks. Consequently, the distribution of communities varied across districts, with the northern region having the highest number (25), followed by the southern region (16), eastern region (12), and western region (7).



A total of 2,740 samples were collected from clinically ill chickens following stakeholder engagement meetings and with the consent of poultry farmers. According to district livestock officials, the sampled chickens had no prior history of vaccination against the disease under investigation. Blood samples of 2–3 ml was collected from the brachial vein using a disposable 5 ml syringe and a 23-gauge needle, and were then transferred into labeled vacutainer tubes. The tubes were allowed to stand overnight at room temperature to facilitate clotting. Subsequently, 1 µl of serum was harvested into cryovials, which were kept on ice packs and stored at the nearest health facility. The samples were then transported to the Njala University Serology and Molecular Laboratory, where they were stored at -50°C until analysis.

Laboratory procedures

Newcastle disease diagnostic procedure: Enzyme-Linked Immunosorbent Assay (ELISA) Test

The Newcastle Disease Virus (NDV-Ab) Antibodies ELISA kit (Svanovir) for the detection of NDV antibodies in serum and egg yolk was used. The kit procedure is based on the blocking enzyme linked immonosorbent assay (Blocking ELISA). All laboratory procedure was follows according to the manufacturer instruction as summarized:

All reagents in the kit were equilibrated to room temperature (18-25 °C). In a 96-well microtiter plate, 100 µl of positive control solution and 100 µl of negative control solution were added to designated wells. To each well, 50 µl of PBS-Tween buffer and 50 µl of undiluted serum sample were then added and mixed thoroughly. The microtiter plates were sealed and incubated at room temperature for 30 minutes. After incubation, the plates were washed three times with PBS-Tween buffer. Subsequently, 100 µl of the conjugate (Horse Radish Peroxidase) was added to each well, and the plates were incubated for an additional 30 minutes at room temperature. Next, 100 µl of substrate solution (tetramethyl-benzidine in substrate buffer containing hydrogen peroxide) was added to each well and incubated for 10 minutes at room temperature. To stop the reaction, 50 µl of stopping solution (0.2M sulfuric acid) was added to each well. The optical densities (OD) of the controls and samples were measured at 450 nm using an ELISA microplate reader.

Newcastle Disease

Data collected for diagnosis of ND were interpreted using the following formula;

$$PI = \frac{(OD_{(Neg\ ctrl)} - OD_{(Sample/pos\ ctrl)} \times 100}{OD_{Neg.ctrl}}$$

Interpretation: PI >40 positive
PI 30-40 Doubtful
PI < 30 Negative

Hemagglutination inhibition (HI) test:

Twenty-five microliters (25 µl) of phosphate-buffered saline (PBS) were added to each well of a V-bottomed 96-well plate. Subsequently, 25 µl of positive serum and 25 µl of negative serum samples were

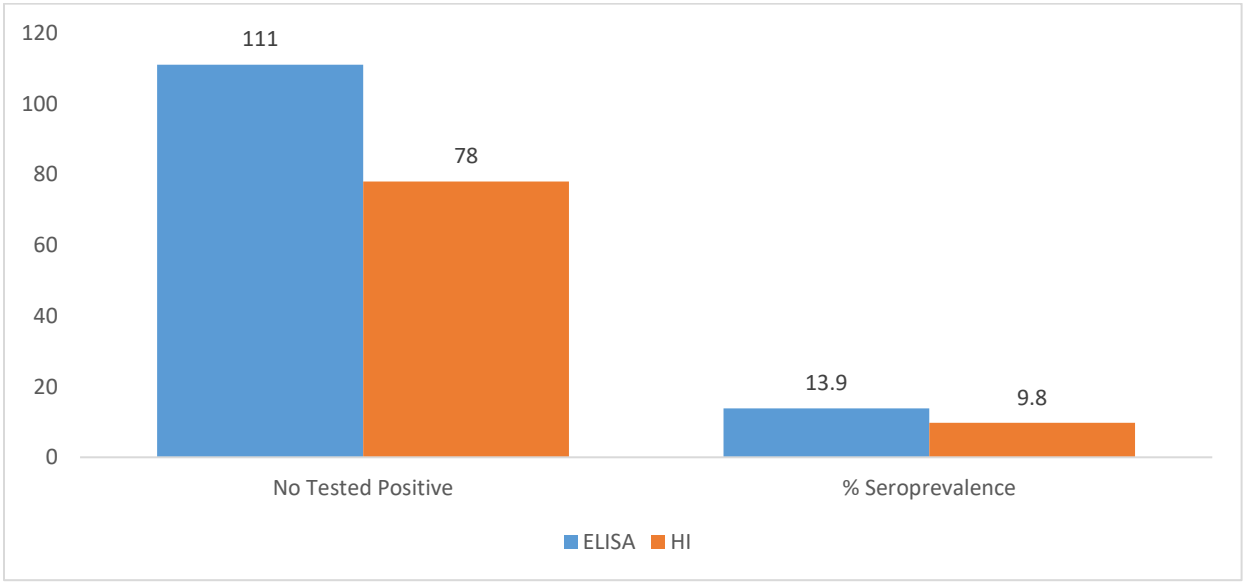
introduced into the first two wells of the first column. The remaining wells were filled with field test sera. Each well was mixed thoroughly, followed by a two-fold serial dilution across the column. Next, 25 µl of standard antigen dilution (4 hemagglutination units, HA) was added to each well, and the plate was incubated at room temperature for 45 minutes. After incubation, 25 µl of a 1% red blood cell (RBC) solution was added to each well, and the plate was incubated for an additional 30 minutes. Results were read and recorded immediately. Only those wells in which the RBCs streamed at the same rate as the control wells were considered positive for hemagglutination inhibition.

Statistical analysis

The data generated from this study were entered into a Microsoft Excel 2016 spreadsheet for coding and cleaning. Subsequently, the data were imported into IBM SPSS version 23 for statistical analysis. To evaluate the strength of association between seroprevalence and the assessed variables—namely sex, age, and location—Pearson's chi-squared test was employed. A p-value of less than 0.05 was considered statistically significant, indicating meaningful relationships among the variables under investigation.

RESULTS AND DISCUSSION

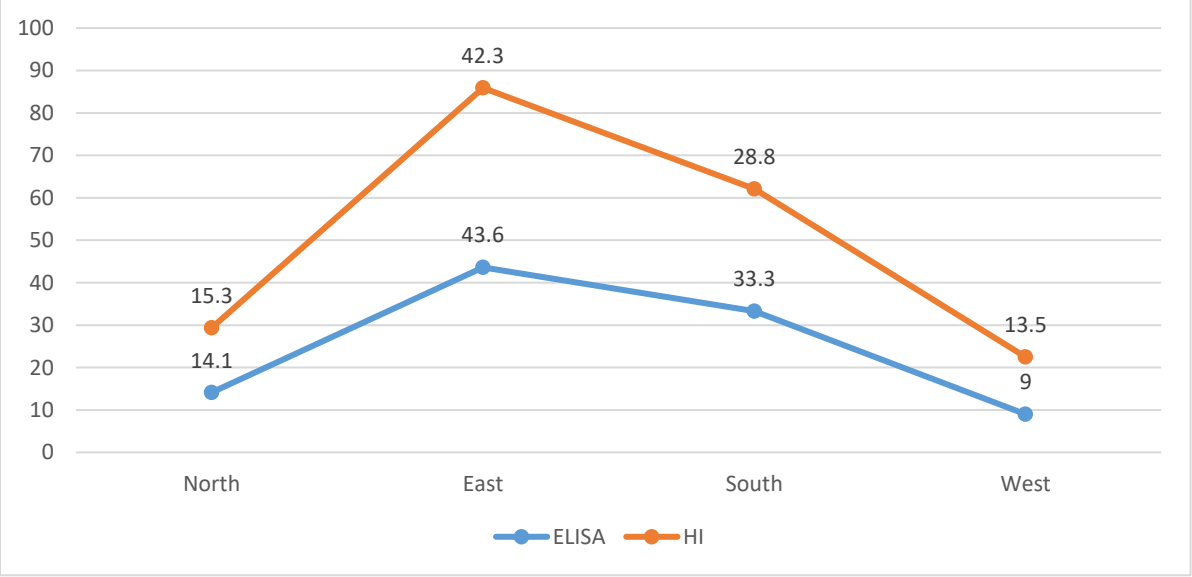
The current study utilized two diagnostic assays, ELISA and hemagglutination inhibition (HI), to assess the prevalence of Newcastle disease among local chickens across various regions of Sierra Leone, while also comparing the sensitivity of these tests in detecting NDV antibodies in clinically infected chickens. Out of 2,740 samples collected, 800 were tested. As illustrated in Figure 2, the competitive ELISA (c-ELISA) identified a higher proportion of positive samples (13.9%) compared to the HI test (9.8%). These results suggest that the ELISA test can be used interchangeably due to its high sensitivity and specificity.



Our findings align with those of [9], who also reported a higher positivity rate with the ELISA test

compared to the HI test. A screening test done by [10] showed superior sensitivity of ELISA test over HI test. A study conducted by [11] revealed a positive correlation between the ELISA and HI tests. However, the overall seropositivity detected in this study using ELISA is relatively lower than estimates from previous studies, including those by [12] in Sierra Leone, [13-14] in Ethiopia and Nigeria respectively. In contrast, [15] reported lower seroprevalence rates than those observed in our study. These discrepancies may reflect variations in regional epidemiology, sampling methods, or the specific populations studied, highlighting the need for continued surveillance and diagnostic evaluations in different contexts.

Figure 1 illustrates the overall seropositivity of Newcastle disease virus (NDV) across four evaluated regions in Sierra Leone. Our study found that the highest seropositivity rates were observed in the eastern region for both diagnostic assays, followed by the southern, northern, and western regions. However, there were notable differences in seropositivity rates between the assays at the regional level. For instance, the northern and western regions exhibited higher seropositivity rates using the hemagglutination inhibition (HI) test (15.3% and 13.5%, respectively) compared to the ELISA test (14.1% and 9.0%). Conversely, the ELISA test revealed greater antibody titers in the eastern and southern regions (43.6% and 33.3%, respectively) than the HI test (42.3% and 28.8%).



The observed variations in antibody positivity rates at the regional level may be attributed to several ecological factors, including differences in vegetation, rainfall patterns, and fluctuations in environmental temperature. Economic factors, such as the live bird trade, along with limited knowledge of biosecurity practices and inadequate veterinary measures, may also contribute to these disparities. Additionally, the presence of migratory wild birds during the sampling period and their interactions with domestic poultry could further influence seropositivity rates. Understanding these factors is crucial for developing targeted control strategies for NDV in Sierra Leone. Table 1 presents the seroprevalence rates of Newcastle disease virus (NDV) in chickens based on various risk factors. Statistically, seropositivity was found to be higher

in male chickens compared to females, with significance noted in the ELISA test ($p < 0.011$), while the HI test did not show significant results ($p > 0.061$). A similar trend was observed concerning age categories; chickens aged one year and older demonstrated the highest susceptibility, whereas those under one year exhibited the lowest seropositivity for both tests (ELISA $p < 0.004$, HI $p > 0.052$).

Variable	No Sampled	Seropositive	% Seropositive	X^2 value	P value		No Sampled	Seropositive	% Seropositive	X^2 value	P value
Sex											
Male	215	41	19.1	7.016	0.011		215	28	13.0	3.58	0.061
Female	585	69	11.8				585	50	8.5		
Age											
< 1 year	381	38	10.0	8.747	0.004		381	29	7.6	3.78	0.052
≥ 1 year	419	72	11.2				419	49	11.7		

Comparable serological studies conducted in the southern region of Sierra Leone corroborated these findings, indicating higher susceptibility in female chickens (Conteh et al. 2020). For instance, research by [16-17 also reported elevated antibody levels in female chickens compared to males. However, contrary to our results, [18] documented higher seropositivity rates in male chickens. The observed differences in seropositivity between the sexes may be attributed to varying exposure levels, particularly as chickens forage for feed and water. Additionally, it is common practice for farmers to retain female chickens as breeding stock, while males are often culled for economic or social reasons, potentially increasing exposure risk for female chickens. This underscores the complex interplay of management practices and ecological factors influencing NDV susceptibility in poultry. Age of chickens were evaluated against ND using the two assays (ELISA and HI tests).

As shown in Table 1, chickens aged one year and older exhibited significantly higher seropositivity rates for Newcastle disease virus (NDV) compared to those under one year, as determined by the ELISA test. In contrast, the hemagglutination inhibition (HI) test did not reveal a statistically significant difference in seropositivity, although younger chickens still displayed the lowest rates. Previous research has established age as a crucial epidemiological factor influencing susceptibility to NDV. For instance, serological studies in Ethiopia conducted by [19-20] reported higher antibody titers in female chickens compared to males. The differences in seropositivity observed in our study may be attributed to increased exposure to NDV in older chickens, coupled with the absence of vaccination history during the study period. This highlights the importance of natural infection in the development of immunity and underscores the need for comprehensive vaccination programs to enhance disease control in poultry populations.

CONCLUSION AND RECOMMENDATIONS

Newcastle disease (ND) presents a significant challenge to poultry production, particularly among local

chicken populations in Sierra Leone. Our study demonstrated that the ELISA test exhibited superior sensitivity in detecting Newcastle disease virus (NDV) compared to the hemagglutination inhibition (HI) test. Additionally, our findings indicated a high seroprevalence of ND, suggesting that the virus is endemic across all provinces in the country. This endemicity poses an ongoing threat to the poultry sector and jeopardizes nutritional security for vulnerable households. In our analysis of risk factors associated with ND occurrence, seropositivity varied significantly by age and sex, with mature and female chickens showing the highest rates when assessed using the ELISA test. However, the overall association between NDV seropositivity and other risk factors did not reach statistical significance. Based on our findings, the ELISA test is recommended as the more suitable diagnostic tool, as it detects a broader range of antibodies against NDV. To mitigate the impact of this disease, it is crucial to implement effective vaccination campaigns at both national and rural levels. Further research is essential to enhance our understanding of NDV epidemiology, including viral isolation, genetic characterization of circulating strains, and the assessment of farmers' knowledge, attitudes, and practices regarding the disease.

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