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Research Article

Sero-Prevalence and Assessment of Presumed Risk Factors on Newcastle Disease of Non-Vaccinated Poultry Flocks

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Abstract

Cross sectional study was conducted on backyard and small scale poultry farms to observe the sero-prevalence of Newcastle Disease (ND) in none vaccinated flocks from March to October, 2021 using an indirect enzyme linked immuno-sorbent assay (ELISA). Of the total 404 serum samples collected for the study, 196 (48.5% (95% CI=43.65-53.41) were positive for the disease antibody detection. An overall mean titer of 5293.65(95% CI; 4687.18-5900.12) was recorded among the sero-positive samples. Four districts were selected for the sampling and of which the likelihood of ND infection was relatively higher for Kindo Koisha district (OR=1.9, 95% CI=0.98-3.59) followed by Sodo Zuria (OR=1.8, 95% CI=0.97-3.35) and Humbo district (OR=1.6, 95% CI=0.82-2.93) as compared to Damot district. However, the mean positive antibody titer at Sodo zuria district was lower (3405.96 ± 318.3393) as compared to other districts. Based on sex, female birds were relatively more likely exposed (OR=1.1, 95% CI=0.74-1.67) as compared to male birds. On the basis of breed type odds of the disease for an exotic breeds were higher (OR=1.4, 95% CI=0.95-2.11) than those produced locally. Significant association (p=0.039) was observed for production system where intensively managed birds (61%) were more prone to the disease (OR=1.8, 95% CI= 1.03-3.18) as compared to an extensively scavenging birds (46.4%). In general, the result finding revealed that a higher distribution of the disease in the study area and likely, in Ethiopia. Further studies to isolate and characterize the circulating NDVs in the study area was highly recommended which enables on the reduction of exposure to the infection with an organized vaccination program and bio-security protocols.

Keywords: ELISA; Newcastle Disease; Non-vaccinated; Seroprevalence; Wolaita Sodo

Introduction

Newcastle disease (ND) is a highly contagious viral disease affecting poultry and wild birds globally [1]. It is regarded as an important reportable poultry disease and a major cause of economic loss in the poultry industry [2]. The causative agent of Newcastle disease virus (NDV), is Avian Paramyxovirus-1 (APMV-1) of the genus Avulavirus belonging to the family of Paramyxovirus serotypes [1].

According to the World Organization for Animal Health, the virulent strains of NDV are responsible for ND infection in poultry [3]. NDV has the ability to infect over 200 species of birds, but the severity of disease produced is dependent on the affected host and the strain of virus [4]. The disease occurs in three pathotypes: Lentogenic, mesogenic and velogenic, reflecting increasing levels of virulence [5]. The most virulent (velogenic) strains are further subdivided into neurotropic and viscerotropic NDVs [5]. In chickens, ND infections are manifested in varying severity ranging from high mortality to silent infection [1]; along with decrease in egg production [6,7]. For instance, the velogenic ND virus induces high mortality reaching 100% in some cases, while other strains such as the mesogenic or lentogenic might elicit severe respiratory disease either by opportunistic infections or in adverse environmental conditions [4].

The transmission of NDV occurs through various ways; of which the role of migratory birds and trade in live birds have been reported as vital routes of ND transmission [8]. Similarly, lack of bio-security, selling of sick birds, exposure to fecal and other excretions from infected birds and contact with contaminated feed, water, equipment and clothing [9] accelerates the transmission of the disease. The disease is characterized by respiratory, nervous, gastrointestinal and reproductive impairment [10,11]. The major mean of prevention against the highly virulent ND is by vaccination, which is achievable with the low pathogenic genotypes attributed to the serological similarity between the NDV genotypes [3].

In Africa and Asia, endemicity of ND remains a significant problem with recent reports suggesting the fast spread of newly identified viruses of sub-genotype into the Middle East [12]. Furthermore, several studies have reported vital risk factors for the transmission of ND in poultry flocks in various countries. The disease is endemic in Ethiopia with frequent outbreaks occurring in commercial poultry flocks and also a threat for those flocks produced at backyard level. Village poultry production is an important economic activity for rural dwellers in the country. Most poultry outbreaks, particularly

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in more remote parts of the country, remain undiagnosed and dead chickens are simply discarded. It is difficult to design and implement chicken health development programs without an understanding of the diseases presence in the backyard poultry production system. ND is among the serious trans-boundary animal diseases (TADs) that cause high mortality and production loss in the majority of our community where at backyard and various production systems.

Generally, based on the devastating effects of NCD and its significant economic importance in the poultry industry, a serological study was conducted on vaccine provision free areas to determine the prevalence of the disease in apparently healthy chicken flocks in four districts of Wolaita sodo zone to generate information necessary to adopt appropriate control measures. Therefore, this study aims to determine the possible risk factors and serological status of the disease in the backyard and small scale flocks in the study area.

Materials and Methods

Study areas

The study was conducted in Wolaita Sodo southern Ethiopia at four selected districts based on variation in geographical location, presence of local/backyard poultry production and ND vaccine delivery free areas were included for the study. Birds aged above two months were included in the study. Damot, Humbo, Kindo Koisha and Sodo Zuria districts were target areas for sample collection. Geographically the areas were located between 6° 53'-7°58" N and37° 46'-37° 58' 40"E with an altitude ranging between 600-2950 meters above sea level [13].

Study design and sampling technique

Cross sectional study was conducted from March to October, 2021.The presence of backyard chickens and small scale production system without history of vaccination were used as an inclusion criterion for the sampling. A stratified sampling method was used to select the number of flocks to be sampled by considering the number of chickens, backyard and small scale farms, and the total number of chickens present in each study districts. In each farm, one healthy chicken were randomly sampled as the flock size in each farms were few. Accordingly, a total of 404 serum samples were collected from backyard (303) and small scaled commercial farms (101). All necessary bio-data's were recorded on the separate data collection formats.

Sampling procedure and sample analysis

Blood samples were collected from the brachial vein in 3-mL disposable syringes and left horizontally for 3 hrs, and then vertically for the serum to ooze out. Serum was decanted into 1.8-mL volume cryovial tubes and kept at -20°C until testing. ND virus antibody works on the principle of indirect nucleoprotein ELISA and is developed to detect specific antibodies against PMV-1 in serum.

Serum samples were analyzed using commercial ELISA kits for the presence of antibodies to NDV (IDVet NDV-Ab ELISA, Veterinary Innovative diagnostic, France), according to the manufacturers' instructions. Briefly, allow all reagents to come into room temperature (21°C + 5°C) before use. Homogenize all reagents by inversion or vortex. The negative and positive controls are supplied ready-to-use and no need of adding dilution buffer to the control wells (A1, B1, C1 and D1). Samples however, are tested at a final dilution of 1:100 in dilution buffer 14 (1:50 pre-dilution, followed by 1:2 dilution in the micro plate). In a pre-dilution plate, set aside wells A1, B1, C1 and D1 for the controls, and add 5µl of each sample to be tested, 245µl of dilution buffer 14 to all wells except control wells. Then, in the ELISA micro plate, add 100µl of the negative control to wells A1 and B1. 100µL of the positive control to wells C1 and D1. 50µl of dilution buffer 14 to each wells except control wells, 50µl of the pre-diluted samples as prepared above. Cover the plate and incubate 30min + 3min at 21°C + 5°C. Prepare the conjugate 1x by diluting the concentrated conjugate 10x to 1:10 in dilution buffer 3. Empty the wells and wash each well 3 times with at least 300µl of the wash solution 1x. Avoid drying of the wells between washings. Add 100µl of the substrate solution to each well and incubate at 21°C + 5°C in the dark for 15min+ 2min. Add 100µl of the stop solution to each well to halt the reaction. The sample and control optical density (OD) values were read using an ELISA reader (ELX800 ELISA Plate reader, BioTek instrument, USA) at 450nm. From OD values, the sample/positive values (S/P) were calculated using the following formula: S/P = ((ODsample- ODnegative control)/ (ODpositive control-ODnegative control) × 100). So, that S/P values < 0.3 were considered negative and S/P values > 0.3 were positive. Similarly, the antibody titer was calculated using the formula; log10 (titer) =1.00x log10 (S/P) +3.520. The antibody titer result can be interpreted as titer < 993 were considered as negative and titer > 993 were positive.

Data analysis

Data from the laboratory analyses were stored in a spread sheet and S/P values were computed using STATA SE/ 13 (Stata Corp., College Station, Texas, USA). Descriptive statistics were computed for all the variables, while the Pearson Chi-square test was used to investigate the association between the sero-prevalence at bird levels in the study areas. Logistic regression analysis was also manipulated to see the effect of one category over the other. A 95% confidence interval with a significance level of 0.05 was used. P- values < 0.05 were considered significant in all attempts of the analysis.

Results

Overall sero-prevalence of ND at the study area

Of the total 404 samples tested for ND indirect ELISA 196 (48.5%; 95% CI: 43.65-53.41) were sero positive for the antibody detection of the disease with a mean titer of 2722.79 (95%CI; 2342.8-3102.78). A mean titer of 5293.65(95%CI; 4687.18-5900.12) was recorded among the sero-positive samples (Table 1).

Distribution of the disease based on geographical location and production system

Based on geographical location various districts exhibit different sero-prevalence for the disease. Indeed, in Damot district the lowest sero-prevalence was recorded (37%) as compared to other districts; Humbo (47.8%), Soddo Zuria (51.5%) and Kindo koisha (52.5%). However, the mean antibody titer for Soddo zuria district was less (3405.96 ± 318.3393) as compared to others as shown in table 2. Within sampling units, small scaled commercial poultry and backyard production system had 61% (36/59) and 46.4% (160/404) of birds with ND antibody positive and mean titers of 3241.6 ± 284.4591and 3761.018 ± 676.8138 consecutively; with statistically

Table 1: Overall sero-prevalence titer values of ND in the current study.

No. of positives (% (95%CI))	Titer values from an overall samples			Titer values from positive samples		
	Mean (95% CI)	Min	Max	Mean (95% CI)	Min	Max
196/404 (48.5 (43.65-53.41))	2722.79 (2342.8-3102.78)	0	15224.38	5293.65 (4687.18-5900.12)	994.77	15224.38

Table 2: Sero prevalence of ND based on geographical location and production system.

Variables	Total sample size	No. of Positives (%)	Mean titer for positives ± SE	χ^2	p-value
District				4.3733	0.224
Damot	62	23 (37%)	5552.902 ± 954.5673		
Humbo	113	54 (47.8%)	6657.954 ± 1035.808		
Kindo Koisha	99	52 (52.5%)	6401.841 ± 1077.033		
Soddo Zuria	130	67 (51.5%)	3405.96 ± 318.3393		
Production System				4.3234	0.038
Small scale	59	36 (61%)	3241.6 ± 284.4591		
Backyard	345	160 (46.4%)	3761.018 ± 676.8138		

Table 3: Sero prevalence of ND based on host related factors.

Variables	Total sample size	No. of Positives (%)	Mean titer for positives (SE)	χ^2	p-value
Flock size				0.0529	0.818
≤ 5 birds	101	50 (49.5%)	5245.818 ± 613.7557		
> 6 birds	303	146 (48.2%)	3825.405 ± 457.0663		
Sex				0.2525	0.615
Male	141	66 (46.8%)	4972.693 ± 617.1612		
Female	263	130(49.4%)	4463.896 ± 586.7669		
Age				1.828	0.401
<3months	77	42 (54.5%)	5196.765 ± 636.2601		
3-6months	155	76 (49.0%)	4575.687 ± 552.1179		
>6months	172	78 (45.3%)	4521.11 ± 588.2155		
Breed				2.9242	0.087
Exotic	164	88 (53.6%)	3554.121 ± 366.2753		
Indigenous	240	108 (45%)	5083.077 ± 727.7038		
Outbreak history				0.8802	0.348
No	279	131 (47%)	4630.46 ± 598.6181		
Yes	125	65 (52%)	5136.192 ± 650.1682		

SE: Standard Error.

significant difference (p=0.038) (Table 2).

Host related factor analysis

Based on flock size the prevalence of ND was 49.5% and 48.2% respectively for those < 5 birds and > 6 birds. Even though the mean titer of positive samples for these categories showed a little bit difference (< 5birds; 5245.818 ± 613.7557 and > 6 birds; 3825.405 ± 457.0663) however, it was statistically not significant (p=0.818). Likewise, the sero-prevalence of the disease showed no significance among sexes of the birds (p > 0.05). Female birds (49.4%) were more infected than males (46.8%) although the mean titer of positive samples was higher for males (4972.693 ± 617.1612) than females (4463.896 ± 586.7669). The mean titer for positive samples in case of age variable was higher for those birds categorized under 3months (5196.765 ± 636.2601) than those found in between 3-6 months age and above 6 months. Similarly, sero-positivity for age groups under 3 months were higher (54.5%) when compared to those aged 3-6 months (49%) and above 6 months (45.3%) although had no significant variation (p > 0.05). According to breed of the birds, exotic breeds were more subjected to the infection (53.6%) than indigenous (45%) with non significant association (p=0.087). However, the positive mean titer of these categories revealed a lower protective immunity development had been observed for exotic (3554.121 ± 366.2753) than local/ indigenous breeds (5083.077 ± 727.7038). Based on the history of NDV outbreak at the study areas, those previously showed an outbreak had higher sero-prevalence (52%) with (5136.192 ± 650.1682) positive mean titer values when compared to those hadn't show an outbreak for the disease (47%) and (4630.46 ± 598.6181) mean titer; with non significant results (p > 0.05) (Table 3).

Univariable logistic regression analysis and interpretation

The risk factors associated with the likelihood of ND infection

Table 4: Effect of covariates over response variable using univariable logistic regression model.

Variables	Categories	No. of Positives (%)	COR (95% CI)	p-value
District	Damot	23 (37%)	*	-
	Humbo	54 (47.8%)	1.6(0.82-2.93)	0.174
	Kindo Koisha	52 (52.5%)	1.9(0.98-3.59)	0.057
	Soddo Zuria	67 (51.5%)	1.8(0.97-3.35)	0.062
Flock size	>6 birds	146 (48.2%)	*	-
	≤ 5 birds	50 (49.5%)	1.1(0.67-1.65)	0.818
Sex	Male	66 (46.8%)	*	-
	Female	130(49.4%)	1.1(0.74-1.67)	0.615
Age	>6 months	78 (45.3%)	*	-
	3-6 months	76 (49.0%)	1.2(0.75-1.79)	0.505
	<3 months	42 (54.5%)	1.4(0.84-2.48)	0.18
Breed	Indigenous	108 (45%)	*	-
	Exotic	88 (53.6%)	1.4(0.95-2.11)	0.088
Outbreak history	No	131 (47%)	*	-
	Yes	65 (52%)	1.2(0.80-1.87)	0.348
Production system	Backyard	160 (46.4%)	*	-
	Small scale	36 (61%)	1.8(1.03-3.18)	0.039

*Reference categories.

was relatively higher for Kindo Koisha district (OR=1.9, 95% CI=0.98-3.59) followed by Sodo Zuria (OR=1.8, 95% CI=0.97-3.35) and Humbo district (OR=1.6, 95% CI=0.82-2.93) as compared to Damot district. Based on sex, female birds were relatively more likely exposed (OR=1.1, 95% CI=0.74-1.67) as compared to male birds. Similarly, the likelihood of infection for birds with a flock size of < 5 (OR=1.1, 95% CI=0.67-1.65) were relatively the same with those having a size of > 6. Likewise, the odds ND infection for birds aged < 3 months were relatively higher (OR=1.4, 95% CI=0.84-2.48) as compared to birds aged 3-6 months (OR=1.2, 95% CI=0.75-1.79) and above 6 months. According to breed type of an avian an exotic breed were more likely affected with the disease (OR=1.4, 95% CI=0.95-2.11) than those produced locally. Significant association (p=0.039) was observed for production system where intensively managed birds were more prone to the disease (OR=1.8, 95% CI= 1.03-3.18) as compared to an extensively scavenging birds. Birds with previous history of NDV outbreak had 1.2 times exposed for the disease than those without an outbreak history (Table 4).

Discussion

New Castle Disease is considered an endemic disease among commercial and backyard poultry farms in Ethiopia. In spite of wide vaccination coverage for the disease there were still frequent outbreak reports from all parts of the country [14]. However, serological tests were still remains as an important immediate detection tools to combat the effect of the disease on the economy. For paramyxo viridea family vaccination is the most and highly practical and cost effective method that ensures successful poultry production via maintaining poultry health at high level [15].

In the present study sero-prevalence of NDV was 48.5%, which is higher compared with the estimates reported in a recent study in West Indies, Trinidad (10%) [16] and Ivory Coast (22%) [17], in a sero-prevalence survey of important viral pathogens affecting backyard chickens. Similarly, in Eastern Showa of Ethiopia, [18] reported a lower sero-prevalence of ND antibody detection (5.9%) [19] In contrast reported a higher NDV sero-prevalence (66%) compared to the current study. The difference might be due to geographical variation, proportions of backyard to commercial poultry farms, degree of previous exposure and management practices.

The sero-prevalence of this study based on geographical location revealed that there is little variation among the study area districts which was much higher than the reports of [18] in Eastern Showa (5.9%). The difference in prevalence could be emanated to the fact that ecological variation for the virus activity and may perhaps be a reflection of the impact of environment on the viability and spread of NDV and its epidemiology [20].

Comparing young and adult cases, most authors observed that ND had a greater chance of occurring in adults. However, infection of birds with ND became increasingly resistant to the disease with age. The low rate of incidence in the adult could be the result of the immunity induced by previous exposure or vaccination [21]. In this study, birds aged below 3months were relatively higher sero-positive than those beyond 3months which was in line to the reports of [22] who reported a higher prevalence in the young/grower (20.7%) against in the adults (12.1%). However, the current result was not in agreement with study of [23] who reported adult birds were more likely infected with NDV (7.45%) than youngest/growers (6.11%). This difference might be observed due to the fact that a continuous cycling of infection between the adult and the young as they scavenge together causes the youngest more susceptible to the disease and the adults were relatively resistant to the infection [21].

With regard to sex, relatively female birds were more sero-prevalent (49.4%) than males (46.8%) in this study. This result was in agreement with the reports of [24] who significantly found higher ND in females (hens) than males (cocks). In contrary to the current result, [25] were found higher seroprevalence of ND in male chickens than in females in Nigeria. Similarly, [26] found a sero positivity of (83.3%) in Ejisu-Besease and (98.4%) in Ejisu-Adumasa for female chickens whereas the males had 100% sero-positivity in Kumasi, Ghana. The variation might be attributed to hormonal difference and stresses related to reproductive activity in female birds which had a predisposing effect more likely to the infection.

On the basis of production system a significant (p=0.039) higher sero-prevalence was observed on the intensively managed birds (61%) than those scavenging extensively (46.4%). This could be due to close relationship to the neighboring birds, restriction from proper areolation, capability for resistivity and environmental factors could influence the epidemiological triad of NDV transmission [19]. Village chickens are naturally resistant and can withstand the infection without showing any clinical symptoms, thus acting as potential source of infection for commercial chicken. This means that village chickens act as host/carrier of NDV; thus, chickens raised in the backyard of farm workers could increase the threat of ND outbreaks [27; 28].

On the basis of breed, Indigenous/local birds were less susceptible

(45%) than exotic breeds (53.6%) with statistically non significant results (p> 0.05). This might be due to the possibility of genetic resistance among indigenous breeds of chickens than exotic breeds, as reported from Egypt [29]; although it is difficult to demonstrate using serological studies, but further studies should be undertaken to investigate.

In general, it is also likely that other infections occur in addition to the ones investigated [30]. As serology is not able to demonstrate which strains are circulating, further work is recommended to better understand the circulating strains or pathotypes and the epidemiology of these diseases. Further study is necessary to understand the interactions of these infectious poultry diseases and to estimate their impact on the backyard poultry production system. Management practices such as disease monitoring program, appropriate prevention, and control measures should be put in place in order to prevent loss of poultry and income due to outbreaks of the disease. New birds should be quarantined and local poultry farmers ensure that they should vaccinate their flocks [27].

Conclusion and Recommendation

Based on these results, the study concludes that a high sero-prevalence of apparently healthy chickens in Wolaita sodo zone, Ethiopia, have been exposed to NDV and this study provided vital data for better understanding of the distribution of ND in the country. This result also explicit that NDV in backyard chickens might have been a potential source of infection to commercial flocks. Further studies should be carried out to isolate and characterize the circulating NDVs in the study area to provide more information that could be used to plan and support for an effective control measures. Factors such as an organized vaccination program, better veterinary services and well performed bio-security measures at poultry farms could assist in minimizing the exposure to Newcastle Disease Virus.

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