

Research Article

Seroprevalence and Associated Risk Factors of Newcastle Disease in Backyard Chicken Production System in Selected Districts of Illubabor Zone, Southwestern Ethiopia

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Abstract

A cross-sectional study was conducted from February 2020 to December 2020 to determine the seroprevalence and associated risk factors of Newcastle disease in selected districts of Illubabor Zone, South-western Ethiopia. Districts were selected by convenient sampling method and PAs were selected randomly. A total of 384 serum samples were collected from chicken of greater than 3 weeks of age and a serological test was conducted using Indirect ELISA. The Indirect ELISA test identified the overall seroprevalence of avian paramyxovirus serotype-1 (APMV-1) 16.93% (65) (95% CI: 13.2-20.7%). This study estimated 12% (95% CI: 5.6-18.4%), 16.8% (95% CI: 10.1-23.5%), and 20% (13.9-26.1%) seroprevalence of Newcastle disease in Hurumu, BiloNopa, and Metu districts respectively. Among the individual chicken risk factors assessed; sex (OR: 2.93, 95% CI: 1.35-6.38, P=0.007) and from flock level risk factors, flock size (OR=1.23, 95% CI=1.006-1.27, P=0.039) and disposal of dead chickens (OR: 11.67, 95% CI: 3.58-38.02, P<0.001) were significantly associated with seroprevalence of Newcastle disease. The results of the present study revealed higher seroprevalence of Newcastle disease in the study area and deserved the implementation of appropriate preventive and control measures and further studies should be undertaken to identify types of strains circulating in this area.

Keywords: Backyard Chicken; Ethiopia; Illubabor; Newcastle Disease; Risk Factors; Seroprevalence

Introduction

Newcastle disease (ND) is an infectious viral disease of domestic poultry and other species of birds regardless of variations in sex and age and is an economically important disease constraint of chicken that inflicts heavy losses both in production and productivity of chicken [1]. It is a viral disease caused by *avianparamyxovirus serotype -1* (APMV-1), which is a single-strand non-segmented negative-sense RNA virus and classified as one of a list A disease of poultry disease according to OIE [2].

The disease probably emerged more than 150 years ago in wild birds and was not recognized as a poultry disease until it was first observed in 1926 in the Indonesia island of Java as chicken disease and it became a severe global problem affecting poultry production [3].

Sources of infection for NDV are exhaled air from infected birds and contaminated feed and water, Feces, eggs during clinical diseases, and all parts of the carcass during acute infection and at death can also act as sources of infection, and transmission is mostly via aerosol. Several risk factors that causes persistence and spread of infection among village chickens includes latently infected carrier chickens, village poultry dynamics (selling, buying, giving), absence of preventive measures, unrestricted contact between other

poultry species (wild birds), flock size, isolation of diseased chicken, and waste/dead bird disposal practice [4]. Accordingly, the role of migratory birds, and trade of live birds were reported as vital routes of ND transmission [5].

Laboratory diagnosis of ND includes virus isolation, serological test (like ELISA, Haemagglutination Inhibition Test), and molecular test like PCR, RT-PCR [6]. ELISA is one of the most widely used bioanalytical methods, where an antigen-antibody reaction occurs and the analyte of interest is detected by an enzyme reporter system [7]. It is characterized by high sensitivity and specificity compared to the microscopic agglutination test (MAT), the gold standard technique. Unlike MAT, ELISA can differentiate between individual immunoglobulin classes and therefore can be used to detect infections in early stages as well as older infections [8].

Even though Ethiopia owned huge number of chickens, management problems and diseases like ND hinders production and productivity of chicken in whole parts of the country. ND is economically the most important chicken disease in Ethiopia due to its regular outbreaks, high mortality and morbidity, difficulty in controlling the disease because of the number of susceptible hosts and implications of the constant variation of the causative virus [4].

Therefore, considering the economic importance of ND, the

absence of formal study in this area, and the contagious nature of the disease, this study was aimed to investigate seroprevalence and associated risk factors of Newcastle disease in backyard chicken production system in selected districts of Illubabor zone, southwestern Ethiopia.

Materials and Methods

Study Area

The present study was conducted in BiloNopa, Hurumu, and Metu districts of Illubabor zone, southwestern Ethiopia (Figure 1). Illubabor zone is located at a latitude of 7°27'40" N and longitude of 34°52'12" E. BiloNopa district has an annual rainfall of around 1692.5mm and 93% of the area is lowland, and 7% is midland and has an average temperature of 24°C with an altitude of 1000-1700 m.a.s.l. The Livestock population of the district comprises 15,591 cattle, 21,351 shoats, 1033 equines, and 89,576 poultry. BiloNopa has sixteen peasant associations in which Abu, Ageta, and Karo were selected for the study of seroprevalence of Newcastle disease [9].

Hurumu is the second district of the study area with mean annual temperature and rain fall of 23°C and 2200mm respectively. The climatic conditions of the area include 86% mid-altitude, 9% low land, and 5% high land. The district owned fourteen peasant associations from which Baro, Sonta, and Toma were selected for the study. The livestock population of this district was 48,395 bovine, 17,359 ovines, 3579 caprines, 61,559 chickens, 695 donkeys, 1159 mule, and 2832 horse [10].

Metu, a market town, is the third study area of the zone with annual rainfall range of 1562-1863mm and temperature range of 18-24°C. The altitude of the district ranges from 1,000 to 2589 m.a.s.l. and populated with 146,635 cattle, 93,012 shoa, 24,372 equine and 103,395 chickens. Three peasant associations (Adale, Boto, and Tulube) were selected for the study from twenty-two peasant associations of Metu district [11].

Study Animals

The target population of the study was comprised of healthy and unvaccinated local backyard chickens clustered at house level. Their age was determined based on history taken from the owners and grouped as young (> 3weeks to < 6 months) and adults (>6 months).

Study Design

A cross-sectional study was conducted from February 2020 to December 2020. The households were clustered based on chickens they have and randomly selected from each PAs.

Sampling Methods and Sample Collection

Districts were selected by convenient sampling method based on proximity to the main road, willingness of respondents to participate and the proportion of chicken; Peasant associations found in the districts were selected randomly. Study chickens were sampled by simple random sampling methods for blood samples collection from the selected households. The sample size was estimated according to the Thrusfield formula using 95% confidence interval, 5% desired absolute precision, and with an assumption of 50% expected prevalence. Accordingly, the sample size was 384 chickens according to the following formula [12].

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

Where, n = sample size, P_{exp} = expected prevalence and d =desired absolute precision (0.05)

The sample size of the interviewee was determined using the formula recommended for survey studies [13].

$$N=0.25/(SE)^2$$

Where: N= sample size, SE= Standard error of the proportion. Assuming the standard error of 5% at a precision level of 5% and 95% CI, so 100 respondents were selected for interview.

Data Collection

The objective of the study was explained to chicken owners verbally and semi-structured questionnaire was used to collect the data. During blood sampling emphasis was given to chicken health determinants like districts and PAs, age, sex, origin, flock size, housing, cleaning the house, uses of disinfectants, sources of water, contact with neighbors chicken, isolation ways of chicken, dead bird disposal and history of vaccination. In the process of data collection 30 (11,8 and 11 from small, medium and large flock containing owners respectively), 26 (11,8 and 7 from small, medium and large flock containing owners respectively) and 44 (21,13 and 10 from small, medium and large flock containing owners respectively) householders were visited from districts of the BiloNopa, Hurumu, and Metu respectively. Out of a total of 52 PAs nine PAs (Abu, Ageta, Karo, Baro, Sonta, Toma, Adale, Boto, and Tulube) were randomly selected from these districts, and backyard chickens were selected by simple random sampling methods from their cluster from the selected households. The average flock size in the study area ranges from 2 to 20 chickens per households, where chickens greater than three weeks were included in the study. According to Win et al.[14], backyard chicken flock can be classified as small (1-7), medium (8-14), and large (> 14), and flock sizes were separated into the small flock (<7) chicken per household, medium flock (<8-<14) chicken per household, and large flock (<14) chicken per household. Of 100 households, two to three chickens from 43 small flock size (total included chickens were 128), four from 29 medium flock size (total included chickens were 116), and five from 28 large flock size (total included chickens were 140) were selected.

Blood Sample Collection

Blood samples of about 2ml were collected from the brachial veins of each chicken using a single use only 3ml syringe and needle. Then samples were put at an angle of 45 degrees for about 20 to 30 minutes, and serums were decanted into cryovial, and transferred to free-transport tubes. Then transported in a cool-flask packed with ice and cotton wool, stored at -20°C until transported to Bedele Regional Veterinary Laboratory and tested using Indirect ELISA to detect antibodies to NDV.

Laboratory Analysis

Indirect ELISA was used for the detection of antibodies against NDV in serum samples using IDvet ID Screen® (IDvet, 310, rue Louis Paster- Grabels -France) at Bedele regional veterinary laboratory, Oromia Region, southwestern Ethiopia. Then all reagents were adapted to room temperature (21°C) before use and homogenized afterward by inversion. The samples were pre-diluted at 1:500 in

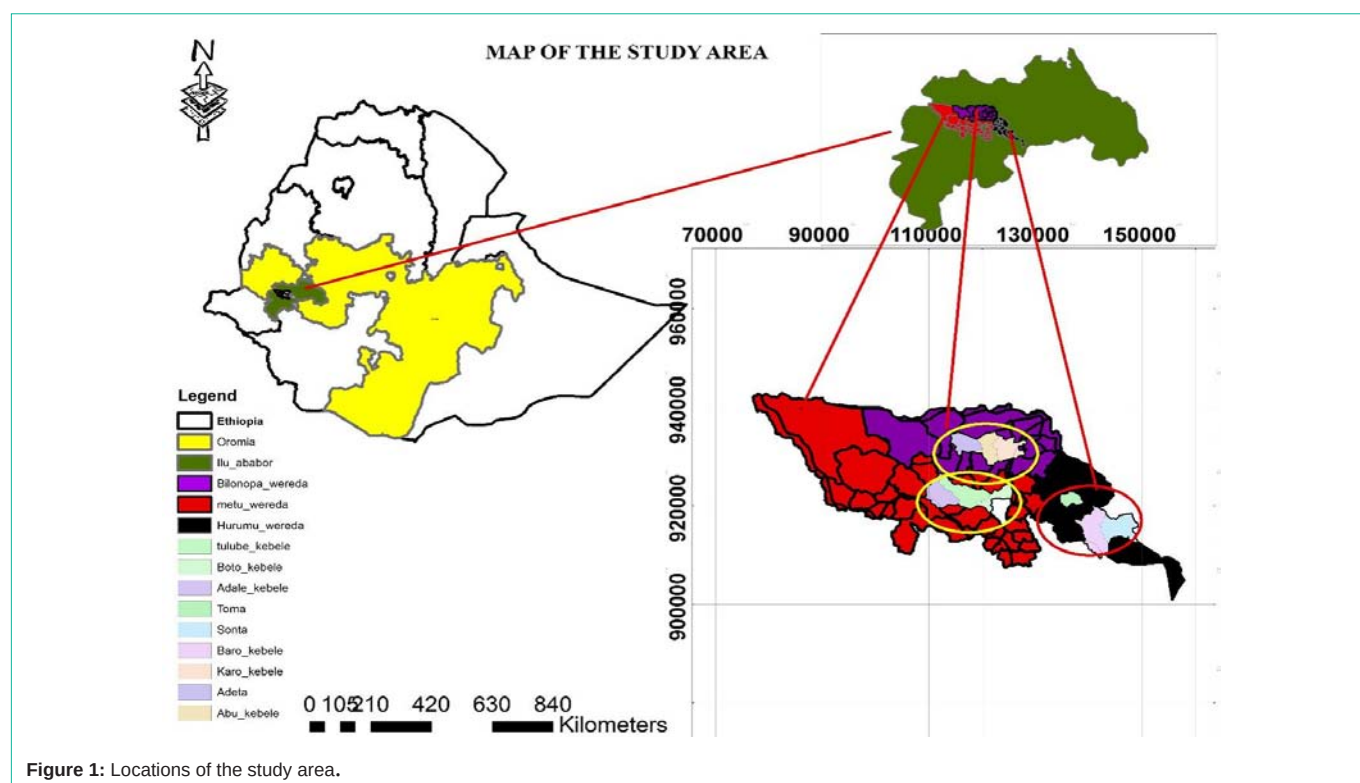


Figure 1: Locations of the study area.

dilution buffer, and a pre-dilution plate of dilution buffer has added. Then the positive and negative control sample has added to the labeled ELISA wells. A 5 μ L of each sample, 90 μ L of dilution buffer, and 10 μ L of prediluted were introduced to the appropriate well of the plate and then covered and incubated for 30 minutes. The conjugates were prepared by diluting concentrated conjugate (Anti-chicken IgG) in a dilution buffer, while the wells were emptied and washed three times with 300 μ L of the washing solution. About 100 μ L of the conjugate reagent was introduced to each well, covered, and incubated as described previously. Again wells were emptied and washed 3 times with 300 μ L of washing solution to remove any unreacted conjugate. Finally, 100 μ L of substrate solution was added to each well, incubated at 21°C for 15 min, and then followed by the addition of 100 μ L of stop solution to halt the reaction. The optical densities (ODs) have been determined by quantifying the absorbance at 405nm using a microplate reader. Also, the sample to the positive ratio (s/p) was calculated and used to determine the mean ratios. Then, the sample was classified into positive and negative based on the comparison of the absorbance between samples and the thresholds defined by the kit's manufacturer.

$$\frac{S/P}{ODPC - ODNC} = \frac{OD \text{ sample} - ODNC}{ODPC - ODNC}$$

Where S/P = Sample to Positive ratio, OD = Optical Density, (ODPC) = Optical density of positive control and ODNC = Optical density of negative control

Data Management and Analysis

All the data collected from the field was recorded in the record sheet format and later entered into a computer and managed using Microsoft Excel worksheet. Then data was edited, coded, and analyzed using STATA software version 13 (STATA Corporation,

4905, Lakeway River, College Station, Texas 77845, USA). Prevalence was calculated for all the data by dividing positive samples to the total number of examined samples and multiplying by 100. Univariate Logistic regression analysis was used to select variables forward for multivariable analysis. The section threshold was $P < 0.25$; where variables with p-value less than 0.25 have been included into multivariable logistic regression analysis. Then multivariable logistic regression analysis was used to estimate the association between explanatory variables (districts, Peasant associations, age, sex, origin (home breed, bought), flock size (small, medium, large) and management systems (like housing, cleaning, uses of disinfectants, source of water, contact isolation, and dead disposal) and seroprevalence of ND (response variable). An odds ratio was the measure of association strength in all variables. In all the analyses, a 95% confidence interval and P-value of less than 0.05 ($P < 0.05$) was set for the significance of statistical associations.

Results

Seroprevalence of Newcastle Disease

Out of 384 chickens selected, 16.93% (65/384) (95% CI: 13.2-20.7) was seropositive for NDV specific antibodies and the overall flock level seroprevalence of NDV specific antibodies was 52% (52/100) (95% CI: 42.2-61.8%) based on at least one seropositive chicken from the flock. The highest seroprevalence (20%) (95% CI: 13.9- 26.1%) was observed in the Metu district and the lowest seroprevalence (12%) (95% CI: 5.6-18.4%) was observed in the Hurumu district. Similarly, the highest seroprevalence (22.5%) (95% CI: 9.6-35.4%) was detected in Ageta peasant association and the lowest seroprevalence (8.6%) (95% CI: 0.7-17.8%) detected in Sonta Peasant Association (Table 1).

Table 1: Seroprevalence of ND from chickens of selected districts of Illubabor zone, Ethiopia.

Variables	Categories	No. of sample	No. of Positive	Prevalence % (95% CI)
Districts	BiloNopa	119	20	16.8 (10.1-23.5)
	Hurumu	100	12	12(5.6 -18.4)
	Metu	165	33	20(13.9 - 26.1)
PAs of BiloNopa	Abu	40	4	10 (0.70 - 19.3)
	Ageta	40	9	22.5 (9.6 -35.4)
	Karo	39	7	17.9 (5.9 -30)
PAs of Hurumu	Baro	34	4	11.8(0.93 -22.6)
	Sonta	35	3	8.6 (0.7-17.8)
	Toma	31	5	16.1 (3.18 - 29)
PAs of Metu	Adale	54	12	22.2 (11.1 -33.3)
	Boto	56	10	17.9 (7.8 -27. 9)
	Tulube	55	11	20 (9.4 -30.6)
Total		384	65	16.93(13.2 -20.7)

Table 2: Univariate logistic regression analysis results of risk factors at chicken level.

Variables	Categories	NCT	NP	Prevalence% (95% CI)	OR (95% CI)	P-Value
Districts	BiloNopa	119	20	16.8(10.1-23.5)	1.48(0.69-3.2)	0.318
	Hurumu*	100	12	12(5.6 -18.4)		
	Metu	165	33	20(13.9 - 26.1)	1.8(0.9-3.74)	0.096
Age	Young	155	16	10.3(5.5-15.1)		
	Adult	229	49	21.4(16.1-26.7)	2.37(1.29-4.34)	0.005
Sex	Male	108	9	8.3(3.1-13.5)		
	Female	276	56	20.3(15.5 - 25)	2.8(1.33-5.89)	0.007
Origin	Home Breed	239	30	12.6(8.4-16.8)		
	Bought	145	35	24.1(17.2-31.1)	2.22(1.29-3.8)	0.004
Contact	Yes	289	50	17.3(12.9-21.7)	0.9(0.48-1.68)	0.733
	No	95	15	15.8(8.5- 23.1)		
Isolation	Yes	98	15	15.3(8.2-22.4)		
	No	286	50	17.5(13.1-21.9)	0.85(0.46-1.6)	0.62

* = Reference Group, NCT= Number of chicken tested, NP= Number of positive, OR =Odds Ratio, CI= Confidence Interval

Individual Chicken Level Analysis

Higher seroprevalence was recorded in adult chicken > 6months 21.4% (95% CI: 16.1-26.7) as compared to young chicken > 3 weeks to <6months 10.3% (95% CI: 5.5-15.1). Similarly, higher seroprevalence was observed in female chickens 20.3% (95% CI: 15.5-25) as compared with male chickens 8.3% (95% CI: 3.1-13.5) and higher seroprevalence in chicken bought from market 24.1% (95% CI: 17.2-31.1) in contrast to chickens hatched in the owners flock 12.6% (95% CI: 8.4-16.8) (Table 2). The result indicated that sex (OR=2.93, 95% CI: 1.35- 6.38, P=0.007) had statistically significant association with seroprevalence of ND at chicken level risk factors analysis (P < 0.05) in which female chicken was 2.93 times more likely seropositive than male chicken (Table 3).

Flock Level Analysis

Relatively higher proportion of seroprevalence of ND was observed in flock whose householders did not disinfect chickens house(54.3%) when compared to owners who disinfect chicken house

(25%); higher seroprevalence in chickens which had contact(53.3%) with neighbors chickens as compared to which did not have contact (48%) with others; the highest seroprevalence in the weekly cleaned house (72.4%) followed by two times cleaned per week (59.3%) and the least seroprevalence were observed in daily cleaned house (34.1%); a higher proportion of ND seroprevalence was observed in householders who did not use disinfectants (59%) as compared to those who used disinfectants (25%) to prevent exposure of chickens to disease and higher in chicken flock in which the owners didn't isolated (54.4%) the diseased chicken as compared to flock in which the owners isolated the diseased one (46.9%) (Table 4).

Flock level risk factors were further analyzed by using a multivariate logistic regression model. Out of flock level risk factors only flock size, uses of disinfectants, and dead disposal system had P-value < 0.25 in univariate logistic regression, and were incorporated into multivariate logistic regression. Multivariate logistic regression analysis shows that flock size (P=0.039) and dead disposal (P<0.001)

Table 3: Multivariate logistic regression analysis of potential risk factors at chicken level.

Risk Factors	Categories	OR	95% CI	P-value
Age	Young			
	Adult	1.73	0.87-3.49	0.12
Sex	Male			
	Female	2.93	1.35-6.38	0.007
Origin	Home breed			
	Bought	1.82	0.97-3.96	0.061

CI: Confidence Interval; OR: Odds Ratio

were statistically significantly associated ($P < 0.05$) with seroprevalence distribution of ND which means that seroprevalence in medium flock size was 0.42 (95% CI 0.12-1.51) times less likely than in small flock size and in large flock size 1.13 more likely than small flock (95% CI: 1.006-1.27) and seroprevalence of ND found in flock scavenging in the area where dead birds disposal in open field was 11.67(95% CI: 3.58-38.02) times more likely seropositive than in flock where dead chickens were buried (Table 5).

Discussion

The present study indicates Newcastle disease as one of the endemic diseases in the study areas of Illubabor zone. Out of 384 serum samples tested by using Indirect ELISA, the overall seroprevalence of ND recorded was 16.93% (65/384) (95% CI: 13.2-20.7%) and 52% (52/100) (95% CI: 42.2-61.8%) at individual chicken level and flock level respectively. As a drawback of the study, indirect ELISA needs an additional incubation step in the procedure and crossreactivity might also arise with the secondary antibody, resulting in nonspecific signal. The study indicated Seroprevalence difference between the

Table 4: Univariate logistic regression analysis results at flock level.

Variables	Categories	NF	NP	Prevalence%(95% CI)	OR(95% CI)	P-Value
Flock size	Small	44	15	34.1(20.1-48.1)	*	
	Medium	27	16	59.3(40.7-77.8)	2.8(1.05 -7.56)	0.04
	Large	29	21	72.4(56.1-88.7)	5.08(1.8-14.15)	0.002
Housing	Share	44	22	50(35.2-64.8)		
	Separate	56	30	53.6(40.5- 66.7)	1.15(0.5-2.5)	0.723
Cleaning	Daily	16	7	43.8(19.4- 68.1)	*	
	Two tmspr week	45	22	48.9(34.3-63.5)	1.23(0.39-3.88)	0.724
	Weekly	39	23	59(43.5-74.4)	1.85 (0.57-5.99)	0.306
Use Disinfectant	Yes	8	2	25(5-55)		
	No	92	50	54.3(44.2-64.5)	3.57(0.68-18.6)	0.131
Water Source	River	63	33	52.4(40- 64.7)	1.04(0.46-2.348)	0.92
	Pond	37	19	51.4(35.2-67.5)		
Contact	Yes	75	40	53.3(42-64.6)	1.24(0.5-3.07)	0.644
	No	25	12	48 (28.4- 67.6)		
Isolation	Yes	32	15	46.9(29.6-64.2)		
	No	68	37	54.4(42.6-66.2)	1.35(0.58-3.14)	0.482
Dead Disposal	Thrown	75	49	65.3(54.6-76.1)	13.82(3.78-50.5)	0.000
	Buried	25	3	12(0.7-24.7)		
Total		100	52	52 (42.2-61.8)	0.99 (0.98-1.01)	0.245

* = Reference Group; NF = Number of Flocks, NP= Number of Positive, OR: Odds Ratio, CI=Confidence Interval, tms = times.

Table 5: Multivariate logistic regression analysis result at flock level.

Risk factors	Categories	St. E	OR	95% CI	P-value
Flock size	Small *				
	Medium	0.654	0.42	0.12-1.51	0.182
	Large	0.066	1.13	1.006-1.27	0.039
Dead dispose	Thrown Buried	7.03	11.67	3.58-38.02	0.000

* = Reference Group; OR= Odds Ratio, CI= Confidence Interval

study districts (BiloNopa, Hurumu, and Metu) which was statistically insignificant ($P < 0.05$). Likewise study result revealed seroprevalence difference of ND between peasant associations. But the difference was statistically not significant ($P < 0.05$). The overall seroprevalence of ND in the study area was interrelated with study results of Zelekeet al. [1] who reported 19.78% from Southern and Rift valley districts of Ethiopia and Gelana [15] reported 12.7% from selected districts of central Ethiopia.

However the present finding was much lower than seroprevalence reported by Tadesseet al. [16] (32.2%) from central Ethiopia, Sonia et al. [17] (97%) from Ecuador; Biswaset al. [18] (88%) from Bangladesh; Jibrilet al. [19] (32.5%) from Zamfara State Nigeria; Geresuet al. [20] (27.86%) from Agerfa and Sinana districts of Bale zone; Getachew et al. [21] (26.2%) from Alamata district, Southern Tigray, Ethiopia; Lawalet al. [22] (62.7%) from Gombe Nigeria, Alsahamiet al. [23] (33.8%) from Oman and Contehet al. [24] (56.4%) from Sierra Leone.

The present study is slightly higher than the seroprevalence reported by Regasaet al. [25] (11%) from Southern Ethiopia, Chaka et al. 4 (5.9%) in Eastern Shewa zone, Terefeet al. [26] (11.6%) from Selected Rift valley areas of Ethiopia, Tilahunet al. [27] (11.34%) from Sebata Hawas district of Ethiopia. The difference in the seroprevalence

of Newcastle disease observed between the reports from different parts of the world and the current study may be due to differences in flock size, sample size, presence or absence of infectious foci which could spread the disease among contact flocks, species of poultry tested and their breeds, ages, diagnostic techniques used, seasonal changes, climatic conditions, and geographical locations [4].

The overall flock level seroprevalence was 52% (95% CI: 42.2-61.8) which was comparable to the seroprevalence of ND reported by Alsahamiet al. [23] (57.1%) at flock level at poultry farms in Oman, Southeastern coast of the Arabian Peninsula in Western Asia. But the current result was higher than seroprevalence reported by Serraoet al. 28 (15.9%) from Timor Leste and Chaka et al. [4] (17.4 %) and (27.4 %) during the wet and dry seasons from Eastern Shewa zone, Ethiopia.

The likely reasons for higher flock level seroprevalence of this findings might be due to poor biosecurity, the introduction of infected chicken into a flock, absence of vaccination, close relationship with neighboring chicken, poor dead disposal, absence of isolation of diseased chicken, and unhygienic feeding practices in backyard chicken production system in the study area. This variation in reported flock seroprevalence between different researchers may be related to the difference in management system like strict biosecurity and use of vaccination, and farming practices like backyard, semi-intensive and commercial production system [1].

This study revealed higher seroprevalence of ND among females as compared to male chicken which had statistically significant association ($P < 0.05$) with seroprevalence of Newcastle disease. This finding was in agreement with the findings of Geresuet al. [20] who reported a higher seroprevalence of ND in females chicken compared to a seroprevalence among male chicken in Agerfa and Sinana Districts, Ethiopia. Also, there was higher seroprevalence of ND reported by Getachew et al. [21] in female (29.2%) than male chicken (15.6%) from Alamata district, Southern Tigray, Ethiopia, Mulalem [29] in female (41%) than male chicken (27%) from selected districts of Arsi Zone, Ethiopia; Tilahunet al. [27] in female chicken (13.5%) as compared to male chicken (6%) in SebetaHawas district, Central Ethiopia.

In contrary to this finding, a study conducted by Zeleke et al. [1] in the Southern and Rift Valley districts of Ethiopia shows a higher seroprevalence rate of ND among males (21.74%) than females (19.16%), and by Jibril et al. [19] with higher prevalence in male (35.5%) than female (28.9%) chicken in Zamfara state of Nigeria.

Therefore, the higher seroprevalence of ND in female chickens in this study area was possibly due to weak immune status of female compared to male chicken which may be as a result of stress during eggs laying and brooding. There is also no additional grains given to return reduction of female chicken weight and energy loss during eggs laying and they were the main flock composition in the study area because they were mostly retained for long periods for egg production purposes compared to the male chicken in which the male was mostly sold during holidays for slaughter purpose which serves the owners as income generation and for buying other materials [4,21].

The present study shows a difference in the seroprevalence between the age groups even though the difference had statistically

no significant associations with seroprevalence of ND ($P > 0.05$). This result reveals adults had a relatively higher seroprevalence of ND 21.4% (95% CI: 16.1-26.7) than young 10.3% (95% CI: 5.5-15.1). This finding was similar to the study reported by Geresuet al. [20] from Agerfa and Sinana districts of Bale Zone, Ethiopia, Getachew et al. [21] from Alamata district, Southern Tigray, Ethiopia and Tilahunet al. [27] from SebetaHa was district of Central Ethiopia.

In contrast to this finding Birhan et al. [30] reported higher prevalence in young chickens (91.19%) as compared to adult chickens (28.6%) from Gondar zone, Ethiopia. This difference in seroprevalence between age groups might be due to more frequent exposure of older chicken to field virus, adult chickens may have higher chance of contact with wild birds than young during scavenging feeds, old chicken might be exposed to ND at an earlier age and most adult chickens examined was purchased from nearby markets which plays a role in their exposure to NDV.

Though there was no statistically significant difference in seroprevalence of Newcastle disease between live chickens purchased from the nearby markets, the results of current study indicated higher seroprevalence in chicken bought/purchased from markets as compared to chicken hatched in farmers own flock or home breed. This finding agrees with the result reported by Jibrilet al. [19] in live bird markets from Zamfara state of Nigeria. This might be because live bird markets contribute to the persistence and spread of ND virus because of their exposure to chicken from multiple sources having a higher tendency of circulating virus and may serve as a source of infection to household chicken as reported by Zelekeet al. [1].

The present study revealed a statistically significant association with seroprevalence of Newcastle disease between different flock sizes of chickens ($p < 0.05$) with the highest seroprevalence in larger flock size 72.4% (95% CI: 72.4-88.7) followed by medium 59.3% (95% CI: 40.7-77.8) and small flock size 34.1% (95% CI: 20.1-48.1). This result agrees with the work of Jarsoet al. [31] who reported a higher seroprevalence of ND in chicken flock size of ≥ 10 and lower seroprevalence of ND in chicken flock size of ≤ 4 from Eastern Shewa Zone, Ethiopia. Similarly, Getachew et al. [21] reported 28.1% in large flock size (6-11 chicken) and 24.1% in small flock (1-5 chicken) from the Alamata district, Southern Tigray, Ethiopia and also seroprevalence of 40% and 38% were reported by Mulualem [29] from flock size of > 10 and (1-10) chicken in selected districts of Arsi Zone, Ethiopia respectively.

This highest seroprevalence reported in larger flock size might be due to that increased chicken number facilitates disease transmission especially when infected chickens entered the large flocks than others and cause widely arising of disease; most chickens in large flocks of this area was purchased from different markets at different time in which concentration of chickens in a single point induces stress and may increases susceptibility of chickens that leads to highest prevalence in the large flock [29].

The results of the present study revealed that dead disposal had a statistically significant association ($P < 0.05$) with seroprevalence of ND in the study area. A higher seroprevalence was reported from the flock management system in which dead chicken was thrown to the external environments like crops cultivation areas where backyard chickens scavenge their feeds 65.3% (95% CI: 54.6-76.1)

as compared to chicken flock where dead chicken were buried 12% (95% CI: 0.7-24.7). This data indicated local chickens kept under free-range traditional management systems that scavenge their feed had easily exposed to ND virus from the simply thrown away of the dead body of chickens and their feces in the field that might create higher seroprevalence of ND in the study area.

In this study risk factors at flock level like housing condition, cleaning frequency, source of water, use of disinfectants, isolation of diseased chicken, and contact with neighbors chicken indicated statistically insignificant association ($P < 0.05$) with the seroprevalence of ND between groups. Even though there was no statistical association between different groups; this study shows slightly higher seroprevalence of ND in chicken kept by mixed with other animals 53.6% (95% CI: 40.5-66.7) as compared to chicken that shared the same house with the owners 50% (95% CI: 35.2-64.8) and highest seroprevalence in the chicken house cleaned weekly 59% (95% CI: 43.5-74.4), followed by the house which was cleaned two times per week 48.9% (95% CI: 34.3-63.5) and lower in daily cleaned house 43.8% (95% CI: 19.4-68.1).

However there was no statistically significant association with seroprevalence of ND; the present result indicated a higher seroprevalence of ND in flock house which was not disinfected 54.3% (95% CI: 44.2-64.5) as compared to disinfected house 25% (95% CI: 5-55); slightly higher seroprevalence ND in the chicken flock that was provided a river as a source of water 52.4% (95% CI: 40-64.7) compared to those provided pond water as a source of water 51.4% (95% CI: 035.2-67.5); higher seroprevalence in the chicken flock which had contact with neighbors chickens 53.3% (95% CI: 42-64.6) compared to a flock which did not mixed with neighbors chickens 48% (95% CI: 28.4-67.6) and there was higher seroprevalence in chicken flock owners who did not isolate 54.4% (95% CI: 42.6-66.2) diseased chicken from their flock as compared to others who isolated 46.9% (95% CI: 29.6-64.2) diseased chickens.

Conclusion and Recommendations

The present study indicated that 16.93% and 52% of detectable antibodies to ND at chicken and flock level which indicated as ND is prevalent in backyard chicken production of BiloNopa, Hurumu and Metu districts of Illubabor zone which may aggravates morbidity, mortality, high risks of NDV transmission, and cause a decrease in production and productivity of chicken. Risk factors like sex, flock size, and management of dead bird disposal was significantly associated with seroprevalence of ND. Therefore the presence of higher seroprevalence of NDV in these chickens was a result of survival from natural infection from field NDV since none of the chickens in the flocks were vaccinated and the chicken was scavenging on free-range. Further investigations are recommended to identify the circulating virus genotypes and models of transmission for better understanding of ND epidemiology in backyard chickens in Ethiopia. Proper disposal of dead chickens and provision of supplementary grains for egg laying chickens were recommended.

References

1. Zeleke A, Sori T, Gelaye E, Ayelet G. Newcastle disease in village chickens in the southern and rift valley districts in Ethiopia. *International Journal of Poultry Science*. 2005; 4(7): 507-10.
2. Worku T, Teshome I. Review on the Role of Viral Structural Proteins on the Pathogenicity of Newcastle Disease Virus in Chickens. *American Journal of Zoology*. 2020; 3(2): 40-6.
3. Arifin MA, Mel M, Salim SH, Karim MI, Hassan SS. Optimization of Newcastle disease virus production in T-flask. *African Journal of Biotechnology*. 2011; 10(81): 18816-23.
4. Chaka H, Goutard F, Gil P, Abolnik C, Almeida RSD, Bisschop S, et al. Serological and molecular investigation of Newcastle disease in household chicken flocks and associated markets in Eastern Shewa zone, Ethiopia. *Tropical Animal Health and Production*. 2012; 45(3): 705-714. doi:10.1007/s11250-012-0278-y.
5. Ayala AJ, Dimitrov KM, Becker CR, Goraichuk IV, Arns CW, Bolotin VI, et al. Presence of Vaccine-Derived Newcastle Disease Viruses in Wild Birds. *PLoS ONE*. 2016; 11(9): e0162484. doi:10.1371/journal.pone.0162484.
6. Ashraf A, Shah MS. Newcastle disease: present status and future challenges for developing countries. *African Journal of Microbiology Research*. 2014; 8(5): 411-6.
7. Pruvot M, Forde TL, Steele J, Kutz SJ, Buck JD, Meer FVD, et al. The modification and evaluation of an ELISA test for the surveillance of *Mycobacterium avium* subsp. *paratuberculosis* infection in wild ruminants. *BMC Veterinary Research*. 2013; 9(1): 5. doi:10.1186/1746-6148-9-5.
8. Desa G, Deneke Y, Begna F, Tolosa T. Seroprevalence and Associated Risk Factors of *Leptospira interrogans* Serogroup Sejroe Serovar Hardjo in Dairy Farms in and around Jimma Town, Southwestern Ethiopia. *Veterinary Medicine International*. 2021; 2021.
9. Bilo Nopa District Agricultural, Livestock and Fishery Development Office (BNDALFDO), 2020.
10. Hurumu District Agricultural, Livestock and Fishery Development Office (HDALFDO), 2020.
11. Metu District Agricultural, Livestock and Fishery Development Office (MDALFDO), 2020.
12. M. Thrusfield. Describing disease occurrence. *Veterinary epidemiology*. 3rd edition. Blackwell Publishing. 2007; 46-74.
13. H. Arsham. Questionnaire design and survey sampling. <http://www.mirror.org/site/hom.ubaltdedu/ntsbarsh/Business-stat>. 2007.
14. Win TTZ, Campbell A, Magalhães RJS, Oo KN, Henning J. Characteristics of Livestock Husbandry and Management Practice in the Central Dry Zone of Myanmar. *Tropical Animal Health and Production*. 2018; 51(3): 643-654. doi:10.1007/s11250-018-1738-9.
15. Esubalew E. Molecular Characterization for Strains of Newcastle Disease Virus Circulating in Chickens in Selected Migratory Birds Abundant Areas in the Mid Rift Valley and Central Part of Ethiopia (Doctoral dissertation, ASTU).
16. Tadesse S, Ashenafi H, Aschalew Z. Seroprevalence study of Newcastle disease in local chickens in central Ethiopia. *Int J Appl Res Vet Med*. 2005; 3(1): 25-9.
17. Hernandez-Divers SM, Villegas P, Prieto F, Unda JC, Stedman N, Ritchie B, Carroll R, Hernandez-Divers SJ. A survey of selected avian pathogens of backyard poultry in northwestern Ecuador. *Journal of Avian Medicine and Surgery*. 2006; 20(3): 147-58.
18. Biswas PK, Barua H, Uddin GMN, Biswas D, Ahad A, Debnath NC. Serosurvey of five viruses in chickens on smallholdings in Bangladesh. *Preventive veterinary medicine*. 2009; 88(1): 67-71. doi:10.1016/j.prevetmed.2008.06.018.
19. Jibril AH, Umoh JU, Kabir J, Saidu L, Magaji AA, Bello MB, Raji AA. Newcastle disease in local chickens of live bird markets and households in Zamfara State, Nigeria. *International Scholarly Research Notices*. 2014;

- 2014.
20. Geresu MA, Elemo KK, Kassa GM. Newcastle disease: Seroprevalence and associated risk factors in backyard and small scale chicken producer farms in Agarfa and Sinana Districts of Bale Zone, Ethiopia. *Journal of Veterinary Medicine and Animal Health*. 2016; 8(8): 99-106.
21. Derbew G, Getachew B, Haftu B. Sero-Prevalence Of Newcastle Disease And Its Associated Risk Factors In Village Chickens At Alamata District, Southern Tigray, Ethiopia. *International Journal of Engineering Development and Research*. 2016; 4(3): 747-52.
22. Lawal JR, El-Yuguda AD, Ibrahim UI. Survey on prevalence of Newcastle disease antibodies in village poultry at live birds markets in Gombe, Nigeria. *J. Anim. Sci. Livest. Prod*. 2016; 1(1): 1.
23. Alsahami A, Ideris A, Omar A, Ramanoon SZ, Sadiq MB. Seroprevalence of Newcastle disease virus in backyard chickens and herd-level risk factors of Newcastle disease in poultry farms in Oman. *International Journal of Veterinary Science and Medicine*. 2018; 6(2): 186-191. doi:10.1016/j.ijvsm.2018.06.004.
24. Conteh AM, Moiforay SK, Sesay ME, Kallon S. Prevalence of Newcastle Disease Virus Antibodies in Apparently Healthy Chickens in Sierra Leone. *Animal and Veterinary Sciences*. 2020; 8(5): 99.
25. Regasa C, Fufa D, Berihamu S, Hunduma D. Seroprevalence of Newcastle disease in backyard chickens in mid Rift Valley of Oromia, Ethiopia. In 12th International conference of the Association of Institutions of Tropical Veterinary Medicine, Montpellier 2007 (Vol. 121).
26. Terefe BT, Gebrewahd TT, Kumar N, Kiros H. Assessment of marketing systems, customer preference and health management of chickens in free range and intensive poultry production systems in Minjar Shenkora District, Amhara Regional State, Ethiopia. *Ethiopian Veterinary Journal*. 2018; 22(2): 14. doi:10.4314/EVJ.V22I2.2.
27. Mamo T, Yimer L. Serological Investigation of Newcastle Disease in Selected Districts of BunoBedelle Zone, Ethiopia. *Veterinary Medicine: Research and Reports*. 2021; 12: 253.
28. Serrão E, Meers J, Pym R, Copland R, Eagles D, Henning J. Prevalence and incidence of Newcastle disease and prevalence of Avian Influenza infection of scavenging village chickens in Timor-Leste. *Preventive veterinary medicine*. 2012; 104(3-4): 301-308. doi:10.1016/j.prevetmed.2011.12.018.
29. Abebe A. Assessment of Production Performance of Improved Chickens Under Rural Management Practices in DugdaWoreda, East-Shewa Zone, Oromia Region, Ethiopia (Doctoral dissertation, Addis Ababa University).
30. Birhan M, Birhan M, Tesfaye S, Tariku A. Detection of antibodies against Newcastle and infectious bursal disease on chicken in north Gondar zone, Ethiopia. *Online Journal of Animal and Feed Research*. 2019; 9(2): 51-8.
31. Jarso DM. Incidence of Village Chicken Diseases in Eastern Shewa Zone, Ethiopia: The Case of Newcastle and Infectious Bursal. *Open Access Journal of Veterinary Science & Research*. 2016; 1: 1-4.