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## New castle Disease: Identifying Sero-prevalence Status and Risk Factors for Small Scale Backyard Chicken Production System in Selected Districts of South Western Shoa Zone

**Moti Wakgari**

Department of Veterinary Epidemiology, Bedelle Regional Veterinary Laboratory Center, P. O. Box 15, Bedelle, Ethiopia

\* Corresponding Author: **Moti Wakgari**

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### Abstract

The study was done on economically very important New castle Disease using cross sectional study from February to April 2022 in selected districts of South Western Shoa Zone in Ethiopia where outbreaks of a disease frequently occurred and sample size was determined using cluster random sampling. So, the objectives of the study were to know the status of sero-prevalence and its risk factors. Serological Indirect ELISA test was used to detect viral antibody and Multivariable Logistic Regression was used for analysis. The result showed that the overall chicken level sero-prevalence was 4.43% (17/384, 95% CI: 2.78 - 6.97) and flock-level seroprevalence 11.20% (14/125, 95% CI: 6.79 – 17.92). Multivariable analysis at chicken level indicated that the odds of Newcastle disease infection was significantly high in cross chicken breeds, in purchased and female chickens. In addition it was high at flock level in cross breed chicken flocks or mixed breed flocks ( $P < 0.05$ ) in compare to local breed flocks. From chicken level risk factors; breed ( $P = 0.019$ ), chicken source ( $P = 0.008$ ), sex ( $P = 0.001$ ) and flock level risk factors, flock mixture ( $P = 0.000$ ) were significantly associated with Newcastle disease infection. The seroprevalence of NCDV in the backyard chicken production system may be due to field exposure of chickens to the disease and revealed the significance of the additional study on the serotype and strains of NCDV that are circulating in the study sites to plan suitable control and prevention actions.

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### 1. Introduction

The world poultry population has been likely to be about 16.2 billion, with 71.6 % in developing countries, producing 67, 718,544 metric tons of chicken meat and 57,861,747 metric tons of hen eggs<sup>[1]</sup>. In Africa, village poultry provides over 70% of poultry products and 20% of animal protein intake<sup>[2]</sup>. In East Africa over 80% of human population live in rural areas and over 75% of these households keep indigenous chickens and Ethiopia is not exception to this situation<sup>[2]</sup>. Recent estimates put the poultry population in Ethiopia at around 56 million with native chicken of none descriptive breeds (local breeds) representing 88%, hybrid chicken 5% and exotic breeds of chickens mainly kept in urban and peri-urban areas 6.45%<sup>[3]</sup>. From the total population of chicken in Ethiopia, 99 % are raised under the traditional back yard system of management, while 1 % is under intensive management system<sup>[4]</sup>.

Since village backyard chickens habitually exposed to overwhelming numbers of microorganisms, Newcastle disease is reported to be the key health and production constraints of chickens<sup>[5]</sup>. Newcastle disease is an acute infectious viral disease of domestic poultry and other species of birds despite of difference in sex and age<sup>[6]</sup>. It is known by respiratory, nervous system impairment, gastrointestinal and reproductive problems. The Newcastle disease virus (NDV) strains are categorized as velogenic, mesogenic, and lentogenic primarily based on their pathotypes and virulence. ND virulent strains are related to intense financial losses because of excessive morbidity and mortality, drop in egg-laying, and lesions in the upper respiratory and digestive tracts<sup>[7]</sup>.

During its transmission sources of infection for NDV are exhaled air from infected birds and infected feed and water and

transmission is frequently via aerosol. Feces, eggs lay during clinical diseases, and all parts of the carcass during acute infection and at death can also act as sources of infection. Chickens infected with virulent NDV may die without showing any clinical sign of illness though young chickens are more susceptible and show sign sooner than older ones. An outbreak of ND is unpredictable and discourage villager from paying proper attention to the husbandry and welfare of their chickens [18]. Hence, this study was conducted to determine the seroprevalence of ND that potentially affect backyard chicken production in study areas and to assess the risk factors contributing to ND seropositivity in the districts.

Consequently, my study could complement the shortage of information about seroprevalence of NDV and associated risk factors in poultry industry sector of the study areas.

## 2. Materials and Methods

### 2.1 Study area

The study was conducted in April 2022 in Wanchi, Ilu and Goro districts of South Western Shoa Zone, Oromia regional state of Ethiopia. This zone is located on the South West of Ethiopia on 100km from Addis Ababa capital city of Ethiopia (Figure 1). The chicken population of Ilu, Wenchi and Goro were 63037, 134685 and 43510 respectively.

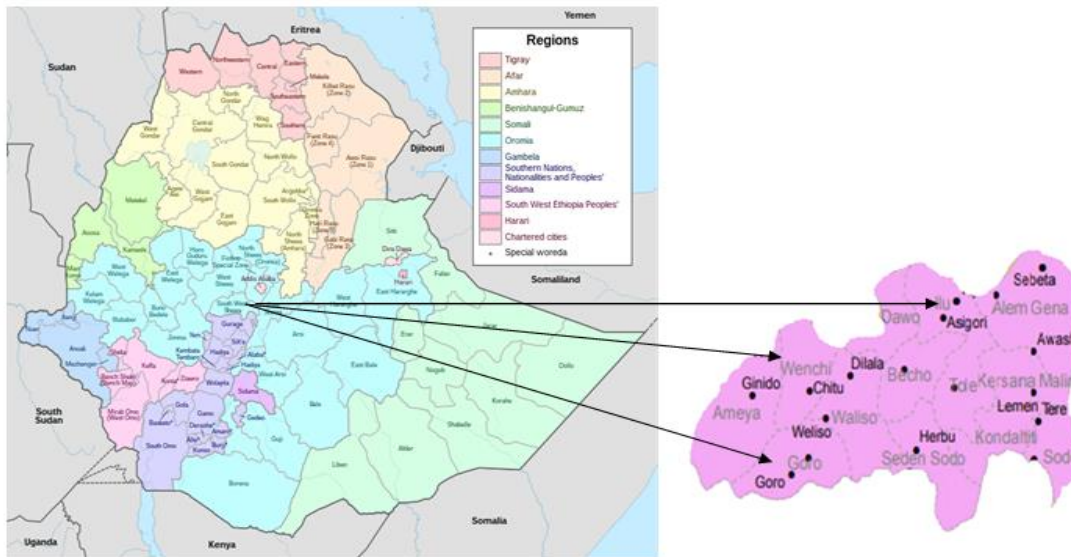


Fig 1: Study area

### 2.2 Study Population and their Management

The study was conducted in chickens of backyard production system of three districts. Most of them were scavenging chickens living together with people and other species of livestock. Many of households bought cross breed chickens and they scavenge with local breeds with grain commonly maize supplement. Most farmers buy cross breed chickens from open markets while few of them buy from small-scale poultry farm micro-enterprises.

### 2.3 Study Design

The study was done in April 2022 in purposively selected districts with randomly selected kebele and using cross sectional study design. Variables included in the study were: study area, breed (local, cross), age (young, adult), and mixture of flock (either local mixed with cross breed or not), sex and source of chickens (home breed, purchased) were emphasized as risk factors.

### 2.4 Sampling Methods and Sample Collection

Districts were chosen purposively due to chicken morbidity and mortality reports of disease in last year but Kebele and household 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 [19].

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

Where, n = sample size, Pexp = expected prevalence and d=desired absolute precision (0.05)

The sample size of the interviewee was determined using the formula recommended for survey studies [10].  $N=0.25/(SE)^2$  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 questionnaire interview.

### 2.5 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. The samples were stayed overnight at room temperature and finally collected into cryovial. Then transported in a cool-flask packed with ice and cotton wool, stored at  $-20^{\circ}\text{C}$  until transported to Bedele Regional Veterinary Laboratory and tested using Indirect ELISA to detect antibodies to NDV.

### 2.6 Laboratory Analysis

Indirect ELISA was used for the detection of antibodies against NDV in serum samples using IDvet ID ScreenR (IDvet, 310, rue Louis Paster- Grabels –France) at Bedele regional veterinary laboratory, Oromia Region, southwestern Ethiopia. Then all reagents were adapted to room temperature ( $21^{\circ}\text{C}$ ) before use and homogenized afterward by inversion.

The samples were pre-diluted at 1:500 in dilution buffer, and a pre-dilution plate of dilution buffer has added. A 5 µL of each sample, 90 µL of dilution buffer, and 10µL 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 µL of the washing solution. 100 µL of the conjugate reagent was added to each well, enclosed, and incubated as described before. Again wells were emptied and washed 3 times with 300 µL of washing solution to remove any un reacted conjugate. Finally, 100 µL of substrate solution was added to each well, incubated at 21°C for 15 min, and then followed by the addition of 100 µL 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.

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

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

### 2.7 Data Management and Analysis

All data obtained from the field was recorded in the record sheet format and later entered into Microsoft Excel worksheet and Binary Logistic Regression for flock level data and multilevel mixed-effects model (Generalized Linear Model

logit) for chicken level data statistics was used to summarize the data by using Stata software version 13. The overall prevalence was calculated by dividing positive samples by the total number of examined samples and multiplied by a hundred. Seroprevalence was categorized into chicken level (sex, age, source, study area) and flock level (study areas, cleaning activity, presence of exotic breeds within the flock, number of chickens per flock and housing system of chickens).

Multivariate logistic regression analysis was used to examine the relationship between the outcome variable (seroprevalence) and the different explanatory variables controlling the possible effect of confounders. The Odds Ratio was used to assess the association between the dependent and independent variables. P-value of less than 0.05 ( $P < 0.05$ ) was set for the significance of statistical associations [11].

## 3. Results

### 3.1. Overall Seroprevalence of IBDV antibody

384 chicken serum samples tested for NCDV antibodies to know chicken level infection, AND 17 samples were positive for NCDV antibody with an overall seroprevalence of 4.43% (95% CI: 2.78, 6.97) in the study area.

### 3.2. District and Village level Chicken Seroprevalence of IBDV Antibody

At individual chicken level the seroprevalence was almost equal in three districts of study areas 5%, 4% and 4.5% in Wanchi, Ilu and Goro districts respectively. The seroprevalence was higher in cross breeds(5%) than local breeds(3.30%), in purchased chickens (5.20%) than home breed chickens (2.60%), in females (5.30%) than males (2%), in adults (4.7%) and young chickens (3.2%) as illustrated in (Table 1).

**Table 1:** Sadarkaa lukkuutti tatamsa'ina dhukkubichaa (Seroprevalence of NCDV antibody)

Risk factors	Category	No. tested	Seropositive (%)	Multivariate	
				AOR (95% CI)	P-value
District	Wanchi	123	6(5.0)	1.3 (0.02 - 0.10)	0.415
	Ilu	127	5(4.0)	1.2(0.01 - 0.09 )	0.537
	Goro	134	6(4.5)	RF	
Breed	Cross	263	13(5.0)	2.3 (0.03 - 0.08)	0.019
	Local	121	4(3.3)	RF	
Chicken source	Purchased	268	14(5.2)	2.7 (0.03 - 0.08)	0.008
	Home breed	116	3(2.6)	RF	
Sex	Female	286	15(5.3)	3 (0.03 - 0.09)	0.001
	Male	98	2(2.0)	RF	
Age	Adult	322	15(4.7)	1.5 (0.02 - 0.07)	0.206
	Young	62	2(3.2)	RF	

AOR = Adjusted Odds Ratio, CI = Confidence Interval, RF = Reference Factor

### 3.3. Flock Level Seroprevalence of IBDV Antibody

Out of 125 flocks tested for NCDV, 14 flocks were found positive for Newcastle disease virus antibody and flock level seroprevalence of NCDV was 11.20% (95%CI: 6.79, 17.92%). Comparatively, the highest flock level

seroprevalence of NCDV was observed in Wanchi district (5/39, 12.80%), followed by Goro district (5/45, 11.10%) and Ilu district (4/41, 9.80%). The seroprevalence was higher in flocks mixed with cross breed chickens (13.2%) than those not mixed (5.90%) as illustrated in (Table 2).

**Table 2:** Flock level seroprevalence of NCDV antibody

Risk factors	Category	No. Household	Sero positive (%)	Multivariate	
District				AOR (95% CI)	P-value
	Wanchi	39	5(12.80)	1.28 (0.06-0.27)	0.107
	Ilu	41	4(9.80)	1.14(0.04 - 0.23)	0.266
Flock Mixture	Goro	45	5(11.10)	RF	
	Mixed	91	12(13.20)	3.8(0.07- 0.20)	0.000
	Local only	34	2(5.90)		

AOR = Adjusted Odds Ratio, CI = Confidence Interval, RF = Reference Factor

In logistic regression analysis, chicken breed ( $P = 0.019$ ), sex ( $P = 0.001$ ) and source of chicken ( $P = 0.008$ ) were independent predictors of NCD infection. The odds of NCD seroprevalence were more likely higher in cross chicken breed than local breeds. The odds of NCD seroprevalence were more likely higher in female chickens than male chickens. The odds of NCD seroprevalence were more likely higher in mixed flocks (local and cross) than none mixed chickens (only local breed flocks). Also the odds of NCD seroprevalence were more likely higher in purchased chickens than hatched chickens. The odds of infection were statistically not significant among 3 different districts as shown in (Table 1 and Table 2).

#### 4. Discussion

The current study showed that the prevalence of ND antibodies in backyard chickens production system was low in the study areas of South West Shoa zone. Total of 384 serum samples tested by using Indirect ELISA, the seroprevalence of ND recorded was 4.43% (17/384) (95% CI: 2.78-6.97%) and 11.20% (14/125) (95% CI: 6.79-17.92%) at individual chicken level and flock level respectively. This study result revealed there was few seroprevalence difference of ND between districts and it was not significant ( $P > 0.05$ ) both at individual and flock level.

The total chicken level sero prevalence 4.43% was lower than that of Giragn *et al.*,<sup>[12]</sup> 16.93%, Mamo and Yimer<sup>[13]</sup> 30%, but higher than that of 2.2% in Mexico Gutierrez-Ruiz *et al.*,<sup>[14]</sup> Current results were in agreement seroprevalence in backyard poultry of 4.8% in Mauritania<sup>[15]</sup>, 4.8% in California McBride *et al.*,<sup>[16]</sup> and 5% in South Africa<sup>[17]</sup>, in Ethiopia 5.6%<sup>[18]</sup>. The overall flock level seroprevalence was 11.10% which was nearly comparable to the seroprevalence of ND reported by Serrao *et al.* (15.9%)<sup>[19]</sup> but lower than that of Giragn *et al.* (52%)<sup>[12]</sup>.

At chicken level source of chicken and breed were statistically significant ( $P < 0.05$ ) that showed cross breeds and purchasing chicken from open market or unknown source were a risk factor to NCD that in agreement with that of<sup>[13]</sup>.

The odds of NCD seroprevalence were more likely higher in mixed flocks (local with cross) than none mixed chickens (only local) that showed significance of specific and practicable strategies and policies to farmers to make them stable on their livelihood. This study also revealed a higher seroprevalence rate among the female (5.3%) compared to male chickens (2%) with statistically significance difference ( $p < 0.05$ ). This result was in agreement with the findings of Minda *et al.*<sup>[20]</sup>; Getachew and Berihu<sup>[21]</sup>. This could be because female chickens were frequently retained for long-term production purposes in relation to the males which were regularly used for non-productive purposes (food, income, and socio-cultural or religious rites). There were no statistical significant difference among three districts which could be

due to similar management and environment of study areas. Also there were no statistical significant difference among study districts and age<sup>[13]</sup>.

#### 5. Conclusion

The current study indicates that the seroprevalence of NCDV was low and chickens are endemically infected with NDV in the study areas which cause economic losses in the livestock sector through indirect losses, morbidity, and mortality of chickens and impair the livelihood of larger poor farmers. Furthermore, the present study demonstrated that the seroprevalence of NCDV in backyard chickens was influenced by breed, source of chicken and flock mixture. The seroprevalence of NCDV in the backyard chicken production system might be due to field exposure of chickens to the disease and indicated the importance of the further study on the serotype and strains of NCDV that are circulating in the study sites to design appropriate control and prevention measures.

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#### Competing Interests

The authors announce that they have no conflicts of interests.

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