

## Sero-Prevalence of Newcastle Disease in Poultry under Backyard System in Villages of Bishoftu Town, Ethiopia

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**Abstract:** A cross sectional study was conducted on 384 chicken raised under traditional management system in four villages of Bishoftu area using Haemagglutination Inhibition (HI) test for the detection of antibodies against Newcastle Disease (ND) virus in serum samples collected from chickens with no history of vaccination and apparently healthy during December 2011 to April 2012. An overall sero-positive rate of 23.4% was recorded in this study. The differences in sero-prevalence however, were not significant ( $p > 0.05$ ) among village, sex, age, flock size, feeding system, source of chicken and contact with wild birds. However, housing system had a significant difference ( $p < 0.05$ ) on sero-prevalence rate of the disease. The fact that most of the chicken originated from village had low HI titer (about 54.4%,  $\leq 2 \log_2$ ) warrant the need to systematic consideration for disease prevention strategy in the village chicken. Detailed further study on ND virus strain identification, a survey of major poultry diseases and improved management packages should be devised.

**Key words:** Hemagglutination inhibition test, newcastle disease, sero-prevalence, village chickens, age

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### INTRODUCTION

The total poultry population in Ethiopia is estimated to be about 58.13 million of which 96.4, 3.06 and 0.53% were reported to be indigenous, hybrid and exotic breeds, respectively. Free-range poultry keeping is most common in the country. The chickens under traditional or “back yard” conditions accounts for 99% while only 1% of birds kept under intensive management system in commercial farms of Ethiopia (CSA, 2004). The contribution of the chicken industry to the national economy and per-capita meat and egg consumption is very low. At the same time poverty and protein deficiency is manifested by wide spread malnutrition in children and women in village communities. The low input might be however, a result of the high risk due to high mortalities experienced in village poultry. Diseases and especially the devastating Newcastle Disease (ND) are perceived to be the main constraints which frustrates any investment in this system. Thus, the potential of the free-range chicken production has not been exploited. Therefore if any

success is to be achieved in improvement for free-ranging chicken production it will inevitably depend on the successful control of major poultry diseases in general and ND in particular (Tadesse *et al.*, 2005).

Newcastle disease is caused by Avian Paramyxovirus serotype 1 (APM-1). It is under the family Paramyxoviridae which possess two surface proteins that are important to the identification and behavior of the virus. The first, Hemagglutinin/Neuraminidase (HN) is important in the attachment and release of the virus from the host cells in addition to its serological identification. The other very important surface protein is the Fusion (F) protein which has a critical role in the pathogenesis of the disease (OIE, 2008). It is an avian paramyxovirus that produces pneumoencephalitis in young chickens and other domesticated and wild birds characterized by respiratory and neurological symptoms, enteritis, hemorrhagic lesions and quite often with high mortality (up to 100%). In human it is an occupational disease limited to workers handling infected birds and may produce inflammation of the conjunctiva (Huang *et al.*,

2003; Brook *et al.*, 2007; Pal, 2007). It is considered the most serious poultry disease throughout the world (Herenda and Franco, 1996).

As village chickens have more contacts with these birds, they may play a role in amplifying the virulence of field virus. Therefore, village chickens are probably very important for survival of ND virus in the environment and may also play an important role in the spread of the virus among industrial poultry flocks (Alexander, 2001). The disease is present in endemic form with frequent outbreaks in different parts of the country and it remains as a constant threat to the backyard poultry (Westbury, 2001). Commercial poultry are routinely vaccinated and the village chickens are not normally vaccinated due to logistic problems and financial reasons (Alexander, 2001). The epidemiology and control of ND has been extensively studied and well documented in commercial poultry system but poorly documented in village poultry. The large difference in management between commercial and village poultry prohibit the transfer of epidemiological data and control program of ND from the commercial sector to the village environment. Thus, successes in attempts to control ND in village poultry have been hardly successful (Alexander, 1997).

The epidemiology of ND in village chicken in Ethiopia is not clearly understood. However, it is the most important reoccurring epidemic every year (Tadesse *et al.*, 2005). Although, ND represents the most sever poultry disease responsible for marked economic losses few studies have been done on chicken in Ethiopia and less attempts were tried in Bishoftu despite the fact that poultry production is common in the area. Furthermore, the actual cause of the widespread epidemic of high mortality in local chickens in various parts of Ethiopia has yet to be identified. Such a wide knowledge gap on ND of local chickens is a hindrance to the implementation of effective disease control measures. The objectives of this study are therefore to determine the sero-prevalence of ND in non-vaccinated apparently healthy village chickens and to assess the potential risk factors in four selected villages of Bishoftu town.

## MATERIALS AND METHODS

**Description of study area:** The study was conducted in Bishoftu, from December 2011 to April 2012. It is located at 9°N and 40°E with an altitude of 1800 m above sea level in the central high lands of Ethiopia at 47 km south east of Addis Ababa. It has annual average rain fall of 1152 mm of which 84% falls down during the long rainy season that extends from June to September and short rainy season from March to May with an average rain fall of 800 mm. The mean annual maximum and minimum temperature

Table 1: Area and age distribution of study chickens

Villages	Males	Females	Total
Hora	32	50	82
Babogaya	51	60	111
Keta	44	60	104
Denbi	37	50	87
Total	164	220	384

are 30.70 and 8.5°C, respectively and the mean relative humidity is 61.3% (CSA, 2004). In addition to its proximity to the capital city Addis Ababa, Bihoftu is one of the most scenic locations in the country with crated lakes which adds beauty to the town. Farmers in the vicinity of the town use a mixed crop-livestock production system and representative agro-ecologies of the country. These agro-climatic zones are inhabited with different plant and animal species (Zelege *et al.*, 2005).

**Study animals and sample size determination:** The total number of chickens required for the study was calculated based on the formula given by Thrusfield (2005) from the selected villages randomly. A precision level of 5 and 95% confidence interval was used to calculate the sample size using the equation:

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

Where:

- N = Required sample size, 1.96 the value of z at 95% confidence interval
- P<sub>exp</sub> = Expected prevalence of Newcastle disease (50%) since no earlier prevalence rate in the study area
- D = Desired absolute precision level at 95% confidence interval

Therefore, a total of 384 samples were calculated as the sample size of the study. A total of 384 apparently healthy chickens were randomly selected from villages of study areas (Table 1) and each sample was given an identification numbers. Then samples were transported to the Ethiopian Institute of Agricultural Research (EIAR) in Bishoftu for examination.

**Study design:** A cross-sectional study design was carried out to collect data on newcastel diseas sero prevalences and assessing the poultry husbandry practices in the study areas.

### Data collection methods

**Questionnaire survey:** Questionnaire survey was conducted to have an overview of poultry diseases history in the study areas. During sample collection

process the owners (farmers) were briefly interviewed about flock size, management (housing and feeding system), source of chicken, contact with wild bird, vaccination history and pedigree of the chicken to exclude hybrid chickens.

**Sera collection and testing:** A total of 1.5-2 mL of blood was collected from the humeral region of the wing vein with a syringe and needle of 3 mL size. The syringe with blood was laid nearly horizontally until the blood clotted. After clotting, the syringe was returned to a vertical inverted position and left overnight to permit the serum to ooze out. The separated serum was transferred into cryovial tubes, labeled and stored at -20°C until the hemagglutination inhibition test was performed to detect antibodies against the ND virus (OIE, 2000).

The Hemagglutination-Inhibition (HI) test was carried out by running two fold dilutions of equal volumes (0.025 mL) of Phosphate Buffered Saline (PBS) and test serum (0.025 mL) in a V-bottomed micro titer plates. Four Haemagglutination Units (HAU) of virus/antigen were added to each well and the plate were left at room temperature for a minimum of 30 min. Finally 0.025 mL of 1% (volume/volume) chicken RBCs was added to each well and after gentle mixing, the RBCs were allowed to settle for about 40 min at room temperature. The HI titer was read from the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 0.025 mL RBCs and 0.5 mL PBS only) were considered to show inhibition. A titer  $\geq 4$  (log to base 2) was taken as positive (OIE, 2000, 2008). According to OIE (2008),  $\log_2 4$  HI titer to NDV was considered sero-positive titer below this was considered as sero-negative.

**Data analysis:** The data collected from study area was coded and recorded in Microsoft Excel spread sheet and then analyzed by using SPSS Version 17. The sero-prevalence was calculated by dividing the number of positive chicken by the total number of chickens examined. Percentage to measure the prevalence of ND was considered significant at  $p < 0.05$  and Chi-square ( $\chi^2$ ) to measure association between prevalence with other variables namely: village, sex, housing system, age, flock size, feeding system, source of chicken and contact with bird were the statistical tool applied.

## RESULTS AND DISCUSSION

About 54.4% had low HI titer ( $< \log_2 2$ ) indicated that the village chickens in such areas have highly susceptible

to the pathogenic NDV infection. According to OIE (2008),  $\log_2 4$  HI titer to NDV was considered sero-positive titer below this was considered as sero-negative. The presence of Newcastle diseases virus antibodies in the serum of 23.4% of the chickens in this study is an indication of earlier exposure of the chickens to the virus (Table 2). The fact that 100% of the owners claimed that there was no vaccine administered to their poultry flock for any of the poultry disease supports this assertion.

In the earlier studies, various workers reported variable sero-prevalence rate in developing countries under back yard system. The current report is lower than the report by Bouzari and Morekani (2006) in Iran, Tariq and Taib (2010) in Iraq and Kashem *et al.* (2011) in Bangladesh but comparable to reports of Tadesse *et al.* (2005) and Zelek *et al.* (2005) from Ethiopia. The

Table 2: Sero-prevalence of Newcastle disease in backyard chickens among the four villages

Village	No. of chicken sera samples tested	HI test positive (prevalence rate)	
		No.	Percentage
Hora	82	20	24.4
Babogaya	111	22	19.8
Keta	104	30	28.8
Denbi	87	18	20.7
Overall	384	90	23.4

$\chi^2$ : 2.913; p-value: 0.405

Table 3: Association of risk factors with sero-prevalence of Newcastle disease in backyard chickens

Risk factors	No. of chicken sera samples tested	HI test positive (prevalence rate)		
		No.	Percentage	p-value
<b>Housing system</b>				
Separate roof	33	3	9.0	0.042
Shared roof	351	87	25.0	
Overall	384	90	23.4	
<b>Age (weeks)</b>				
2-21	101	28	27.7	0.425
22-31	240	54	22.5	
32-42	43	8	18.6	
Overall	384	90	23.4	
<b>Flock size</b>				
2-6	143	38	26.6	0.53
7-12	207	45	21.7	
13-18	34	7	20.6	
Overall	384	90	23.4	
<b>Feeding system</b>				
Scavenging	72	17	23.6	0.969
Regular feeding	312	73	23.4	
Overall	384	90	23.4	
<b>Source of chicken</b>				
Purchase	74	22	29.7	0.155
Own	310	68	21.9	
Overall	384	90	23.4	
<b>Contact with wild bird</b>				
No	59	13	22.0	0.782
Yes	325	77	23.6	
Overall	384	90	23.4	

Table 4: Questioner survey results of 57 household respondents in four villages of Bishoftu town

Questions raised during the interview	Number of respondents (n = 57)				HI test positive (prevalence rate) (%)			
	H (13)	B (15)	K (16)	D (13)	H	B	K	D
<b>Flock population</b>								
2-6	7	9	7	6	32.1	28.0	26.5	16.7
7-12	5	6	7	6	16.3	14.0	33.8	22.4
13-18	1	0	2	1	36.4	0.0	0.0	21.4
<b>Separate house for chickens</b>								
Yes	1	0	2	2	10.0	0.0	0.0	6.7
No	12	15	14	11	11.7	20.0	28.8	23.6
<b>Regular feeding for chickens</b>								
Yes	11	12	12	10	24.0	22.0	20.0	17.5
No	2	3	4	3	28.6	18.2	32.4	29.2
<b>Source of chickens</b>								
Own	9	12	12	8	21.9	18.1	26.5	21.7
Purchased	4	3	4	5	33.3	29.4	38.0	16.7
<b>Contact of chicken with wild bird</b>								
Yes	1	0	1	5	0.0	0.0	0.0	25.0
No	12	15	15	8	24.4	19.8	30.9	14.3

H = Hora; B = Babogaya; K = Keta; D = Denbi

discrepancies among the different reports might be attributed to different stages of infection in the population at the time of sampling, sampling strategy, season of study and/or due to a lesser extent to the variability in the result of the test as stated by Alexander (1988) and unsatisfactory reproducibility of HI test. On the other hand, management system in traditional production may serve as a stress factor and favor infection. Poor sanitary conditions, continuous exposure of chickens to range conditions and wild birds, nutritional deficiencies, the absence of vaccination in traditionally managed chickens and contact of chickens of one village with those in other villages may facilitate the spread of ND (Miguel *et al.*, 2013).

There was no significant difference ( $p > 0.05$ ) in sero-prevalence between villages. This may be associated with the fact that the study was conducted in the area having the same agro climatic condition. However, higher positive HI titers was recorded in Keta village 28.85% than others village. There was a significant difference ( $p < 0.05$ ) in sero-prevalence between different housing system. The highest prevalence was recorded in Shared roof (25%) as clearly presented in Table 3. The type size and material of the shelters differed between regions and within the same village. The type and size of shelter was not taken in to consideration. Some of the shelters had raised floors while others had floors at ground level. In questionnaire survey, the farmers claimed to have experienced poultry disease in his/her flocks of chicken with high mortality and found as well positive with HI test which was low 23.4% (Table 4). This could be due to the killer disease claimed to be ND might not necessarily be ND or the animal brought to the market for sell might not be among those affected and recovered one or the farmers provided unfounded information in expectation of some sort of

support. The ease of contact of chickens from different areas at local open air markets which are then taken back to various localities can undoubtedly facilitate the rapid spread and persistence of NCD among local chickens.

## CONCLUSION

The serological evidence of the present investigation showed that there is continuous infection resulting in to endemic nature of the disease. Vast majority of chicken population in the study area had low level of HI titer for ND signifies they are highly susceptible to the pathogenic NDV infection. This demands some sort of preventive strategy using appropriate and effective vaccine under rural setting in sustainable way by consideration of different factors including hygienic condition of the flock, nutritional status, the presence and absence of concurrent infection, good vaccination program and monitoring of vaccination response.

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