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Incidence of Village Chicken Diseases in Eastern Shewa Zone, Ethiopia: The Case of Newcastle and Infectious Bursal Disease

Jarso DM*

Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia

***Corresponding author:** Desalegn Jarso Modjo, Addis Ababa University, College of Veterinary Medicine and Agriculture, Ethiopia, Email: djmojo74.ethio@mail.com

Research Article

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Abstract

A longitudinal study was carried out from September 2014 to May 2015 on village chicken of Lume district for the aim of determining incidence rate of mortality of Newcastle disease (NCD) and infectious bursal disease (IBD) and the associated risk factors. In addition in a retrospective survey past occurrence of these disease was assessed. Simple random sampling method was used to select the peasant associations (PAs) and the households. Owners and veterinary field workers perception on chicken diseases was collected from 120 respondents through structured questionnaire. The majority (75%) of the respondents put diseases as major causes of village chicken mortality, out of which 78.3% of the respondents indicated NCD locally known as "Fangle" as the leading disease that cause mortality of chicken in the village.Of the 1358 registered chicken, 202 (14.9%) survived the entire follow-up period. A total of 843 chickens found dead of NCD outbreak during the follow-up period. The general mortality rate was 62.1% whereas the incidence rate was113.2 cases per 1000 chicken month.Over the duration of the study, serum samples of 521 chickens were collected to confirm the cause of the outbreak, 242 from sick and 279 from apparently health chicken. Serology using HAI and I-ELISA test were conducted to determine the seroprevalence of NCD and IBD, respectively. In total 28.6% (149/521) and 20.7% (108/521) were positive for NCD and IBD, respectively. Among the 242 sera collected from clinically diseased chicken 61.6% (149/242) and 38.4% (93/242) were positive for NCD and IBD, respectively.Statistically significant ($p<0.05$) difference in prevalence of NCD was found between highland and lowland; chicken flock size and sampling months. Statistically significant ($p<0.05$) difference in seroprevalence of IBD was found between different age groups; household flock size and sampling months. This study has shown that NCD and IBD are one of the major infectious diseases threatening the survival and productivity of traditionally managed local chickens in East Showa zone. Thus, routine vaccination program is recommended.

Keywords: Incidence rate; Lume district; Seroprevalen; Survivalanalysis

Introduction

Alarming poverty has been reported in Ethiopia with food and financial crisis. Poultry is an interesting tool to respond rapidly to poverty gaps if included in rural development strategies. It has fast generation interval and high reproductive rate. It is prolific, easy to rear and their output can be generally expanded more rapidly and easily than that of other livestock. Different scales of poultry productions are available in Ethiopia: scavenging, large, small-scale and commercial. The 3 production systems have their own specific chicken breeds, inputs and production properties. Each can sustainably co-exist and contribute to solve the socio-economic problems of different target societies [1].

Chicken production under backyard system has long been practiced in Ethiopia and almost every rural family owns which has been widely used for egg, meat production, other purposes [2,3]. Village chickens contribute more than 98% of the total meat and egg production in the country [4]. The total chicken population in Ethiopia is estimated to be 50.38 million out of which 97% is indigenous breed that are well adapted to the local environmental conditions (hot, humid, dry and rainy weather, feed and disease challenges [5]. The majority (97%) of these chickens are maintained under this scavenging production system with no inputs for health care (CSA, 2010)[6]. In fact, 80% of the total poultry population in the world is in traditional village-based production systems, being ‘‘low input_/low output’’ systems. They have deep-rooted impact in the socio-cultural and economic profile of the rural community. However, in research, extension and development agenda the village indigenous chickens are poorly considered. The commercial poultry sector which covers only approximately 3% is distributed in a limited urban and pri-urban location in Ethiopia, as it demands electricity, infrastructure and investment for intensification. It is found at an infant stage. It is constrained by high cost of input supplies such as day-old exotic chicks and feed [1,6].

Some published information on the constraints to backyard chicken production in Ethiopia indicated, it is characterized by high mortality caused by disease, predators, and poor management and nutrition. Out of which, infectious diseases are one of the most important cause of mortality in village chicken [7-9]. The most devastating diseases of village chicken in Ethiopia are Newcastle disease (NCD) and Infectious Bursal Disease (IBD) [10-13]. An overall 50%and 32.7% mortality rates caused by NCD and IBD were reported by [14] and [13], respectively. The high mortality rate caused by NCD and IBD make the diseases compulsory to get priority over the other diseases. Numbers of works have been published on the seroprevalence of NCD and IBD in village chicken population. Despite the fact that the seroprevalence of NCD and IBD is increasing at an alarming rate all over Ethiopia, no works has been done so far towards estimating incidence of mortality and morbidity and identifying the associated risk factors in East Showa zone via follow up and sero-epidemiology methods. Therefore, objectives of this study were to investigate incidence of morbidity and mortality of NCD and IBD in Village chicken and associated risk factors.

Material and Methods

Description of the Study Area

The study was conducted in East Showa zone of Oromia regional state from September 2014 to May 2015. East Showa zone is located at about 98 km east of Addis Ababa that covers the total area of approximately 10241 Km^2 and Adama town is the capital of the zone. The Zone extends between 70’33’50”N – 90’08’56”N and from 380’24’10”E – 400’ 05’ 34”E. The temperature of the area ranges from 10^0c in uplands to over 30^0c in rift valley depressions with the mean temperature of 20^oC. Since the large portion of the zone is located along the rift valley system, rainfall varies from 600mm to 1000mm with mean annual rainfall of 816 mm. The livestock population of East Showa zone is estimated to be 1,090,091cattle, 319,598 sheep, 568,761 goat, 10644 horse, 7039 mule, 284, 583 donkey, 6818 camels, 14627 beehives and 1,250, 059 poultry [5]. Out of a total 1,250,059 poultry, around 94% (1,169,710) are indigenous poultry whereas only 6% (69,562) are hybrids [5].

The study was conducted in Lume District, which is one of the districts in East Showa Zone. The District covers an area of 92, 751.33 ha. Modjo is the town of the study district, located at 70 km South East of Addis Ababa with a human population of about 95,000. The average altitude is about 1880 meter above sea level. The average annual rainfall is about 839 mm and the average temperature is 24°C. The District has a village poultry population of 24,045 [15]. The soil and climate are similar to many highlands in Ethiopia. Poultry keeping is widely practiced in most rural and urban of Lume district. The area is assumed to be suitable which gave a characteristic climatic condition that is conducive for the production of chicken. Furthermore, due to the geographical proximity of the zone to capital city Addis Ababa, it has a great advantage for market access for poultry products.

Study Design

A prospective study was carried out from September 2014 to May 2015 on village chicken of Lume district for the aim of determining incidence of NCD and IBD and their associated morbidity and mortality. In addition a retrospective survey past occurrence of these disease was assessed by recall methods. Random sampling technique was used to select 6 out of 35 PA found in the district. Then, a list of farm households was prepared jointly with the community representatives, village leaders, village elders and the development agents working in the selected PA's. Finally, simple random sampling technique was employed to select 20 households from each PA, which made a total of 120 households. All chicken in a farm household was sampled as a cluster. A total of 1358 chickens from these 120 household were included in the study. The average flock size per house hold was 11.3. The sample population was unvaccinated apparently health and sick backyard chickens population of all age and sex group found in different PAs of the district.

Methods of Data Collection and Procedures

Questionnaire survey: Questionnaire survey was conducted to gather owner's and veterinary field professional's knowledge of chicken diseases. In all study PAs veterinary personnel and poultry owners were interviewed with a structured questionnaire. Emphasis was given on the frequent clinical symptoms manifested; possible source of the disease; season of the year the disease commonly occurs; more affected chicken groups and history of vaccination, whenever outbreaks of poultry diseases occurred in the study PAs. Tentative diagnosis was made based on the classical disease manifestation and the epidemiological information available. A total of 120 respondents were set for the interview and to follow up their chicken throughout the study period. The respondents were provided with variables such as flock size change, major causes of chicken mortality, date of outbreaks, major disease responsible for the mortality of chicken, seasonality of the diseases, and relation of diseases occurrence with chicken market turnover.

Follow up data collection: A prospective study was conducted to determine the incidence rate, survival rate and predictors of NCD in village chicken death during the nine months (September 2014 to May 2015) of follow-up period. Chicken were visited every week and also visits were made upon argent telephone call. Records were made on chicken flock size dynamics, disease outbreaks, clinical findings and serum sample collection. Formats was prepared for recording of monthly chicken population dynamics and health status of local chickens enable to determine aspects like the incidence of diseases, mortality and morbidity rate, symptoms of the disease and season of occurrence. Data were extracted from the chicken follow up records by investigator and animal health professionals working in the PA clinics of the district. To ensure quality of the collected data one day orientation was given by the investigator to the animal health professionals, Development agents (DAs) and chicken owner. Regular visit and telephone call by the owner of the chicken, animal health professionals and DAs was the main means of communication whenever any morbidity and mortality of chicken were occurred.

Laboratory investigation: Based on congregated epidemiological information, laboratory investigation of causes and determinants of morbidity and mortality of village chicken was made. Apparently health and sick chicken were observed and sample was collected. Then, determining prevalence of ND and IBD virus was done.

Blood sample collection: Blood sample was collected from the brachial vein in 3-mL disposable syringes, left horizontally for 3hr, and then vertically for the serum to ooze out. Serum was collected in labeled 2-mL cryovial tubes and kept cool for transportation to National Animal Heath Diagnostic and and Investigation Center (NAHDIC), Sebata and National Veterinary Institute (NVI). The serum in the cryovial tubes was stored at −20°C until testing. Serum samples were analyzed using Indirect ELISA for IBD and HAI test for ND.

Serology test: Serum samples were analyzed at NAHDIC and NVI, using Hemagglutination Inhibition (HAI) test for ND and Indirect ELISA for IBD.

Indirect ELISA: ID vet innovative diagnostic indirect ELISA kit (Louis Pasteure-Grabels, France) was used to detect the presence of anti-IBD antibodies in the chicken serum following the kit manufacturers' recommended protocol. The test sera were pre-diluted by dilution buffer 14 in a pre-dilution plate according to the established protocol or kit instructions, and each was dispensed into the requested number of micro wells. In the ELISA plate pre-diluted samples and dilution buffer 14 were added and incubated for 30min ± 3min at 21°C. After incubation, the sera were discarded from the plates, and each well was washed 3 times by 300µl of washing solution. About 100µl anti-chicken immunoglobulins peroxidase conjugate was dispensed into the wells and the plates were incubated for 30min ± 3min at 21°C. After incubation, again the sera were discarded from the plates, and each well was washed 3 times by 300µl of washing solution. About 100µl substrate solutions were dispensed into each test well and again incubated for 15 min ± 2min at 21°C in the dark place. After a final incubation, the substrate chromogen reaction was stopped by adding about 100µl stop solution and the color reactions were quantified by measuring the optical density of each well at 450 nm. To check the validity of IBD ELISA result, validity test was done. In valid IBD ELISA result, the mean Optical Density (OD) value of positive control serum is greater than 0.250, and the ratio of the mean value of the positive and negative control (OD_{PC} and OD_{NC}) is greater than 3. For the interpretation of the result, serum sample positive (SP) control ratio was required. Accordingly, the following equation was applied. If SP value was ≥0.3, the IBD antibody status was considered to be positive but <0.3 was taken as negative.

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

HAI test: was done according to the procedures of NAHDIC (2009). The test was carried out by running two fold dilutions of equal volumes (25µl) of PBS and test serum (25µl) in a V-bottomed micro titer plates. Four HAU of virus/antigen were added to each well and the plate was left at room temperature for a minimum of 30 minutes. Finally 25µl of 1% (v/v) chicken RBCs was added to each well and, after gentle mixing, the RBCs were allowed to settle for about 30 minutes 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 25µl RBCs and 50µl PBS only) were considered to show inhibition. A titter greater than or equal to 2^3 or 3 (log to base 2) was taken as positive.

Data Analysis

Data obtained from Questionnaire, follow up and Laboratory test (HAI and Indirect ELISA) were inserted into Microsoft® Excel for Windows 2007. Analyzes were performed using SPSS statistics 20 software (2011). Survival curve over the follow up time was calculated using the Kaplan Meier and Cox Proportional Hazard Analysis method. Descriptive statistical methods that measures of variability include the variance were used to summarize prevalence of IBD and NCD during outbreaks, and to summarize the population characteristics of the study animals. Chi-square was used to test the presence of significant variation among the different risk factors. Univariate and multivariate logistic regression was conducted to examine the association of the risk factors with occurrence of NCD and IBD. A 95 % confidence intervals were calculated and alpha value of <0.05 was used as cut-off or for significance.

Results

Questionnaire Survey

Flock size change and major causes of chicken mortality: as to chicken flock size, 95.8% (115/120) of the respondents indicated that flock size of chicken was decreased during the last 5 months while 2.5% (3/120) of the respondents indicated that flock size of chicken was increased during the last 5 months. The rest 1.7% (2/120) respondents indicated that flock size of chicken was constant during the last 5 months (Table 1).

In this study, 75% (90/120), 20% (24/120) and 5% (6/120) of the respondents indicated that the higher death of their chicken was due to diseases, predation and unknown cases, respectively. Specific chicken diseases that lead to high mortality were also mentioned by the respondents. Most of the respondents were familiar with NCD locally known as “Fengel” which was manifested by frequent clinical symptoms (greenish dropping, swelling of eyelid with abnormal accumulation of liquid, black comb and brachial vein, lowering the head down, paralysis and sudden death) during disease outbreak. Overall, 78.3% (94/120) of the respondents indicated that “Fengel” was the leading disease to cause mortality of their chicken in the village. While, 15.8% (19/120) of the respondents indicated that Fowl pox locally known as “Fentata” which has frequent clinical symptoms (nodules on the wattles comb and face) was the leading disease to cause chicken mortality. The other 5.8% (7/120) of the respondents indicated that Marek’s disease which was manifested by symptoms like paralysis of wing, dropping of limb and twisted neck was the leading cause of mortality. In relation to season of the year when the diseases frequently occur, most of the respondents (80.8%) indicated, disease occurrence was higher at dry season. However, 10% of the respondents experienced high rate of disease occurrence at the wet season. There were also respondents (9.2%) who experienced disease occurrence at any time in a year. Most of the respondents (95%) indicated that the occurrence of village chicken disease was highly related with high market turnover, especially during holyday celebration. And the rest 5% of the respondents indicated disease occurrence was not related with market turnover, shown in (Table 1).

Variables	Level	No. of respondents	Percent (%)
Chicken flock size change	Decreased	115	95.8
	Increased	3	2.5
	Constant	2	1.7
Causes of mortality	Diseases	90	75
	Predation	24	20
	Unknown	6	5
Major diseases	ND(Fangle)	94	78.3
	Fowl Pox(Fentata)	19	15.8
	MD	7	5.8
Seasonal Loss	Dry Season	97	80.8
	Wet Season	12	10
	Both Season	11	9.2
Chicken off take	High	114	95
	Low	6	5
History of Vaccination	Not vaccinated	119	99.17
	Vaccinated	1	0.83

Table 1: Answers of 120 respondents on different questions

Monthly average number of chicken ownership dynamics: Male chickens suffered from population reduction in different months than the female counterparts, because, male chicken were slaughtered for home consumption during holyday and other purpose, given as a gift, sold for household income ahead of diseases outbreak occurrence. Female population reduced in only December within 5 months of follow up. Similarly, chicken population reduced in November, December and January within 5 month follow up. Female and chick population reduction was more related with diseases outbreak occurrence. NCD Disease outbreak was occurred in November, December and January during follow up period (Table 2).

Chicken category	September	October	November	December	January
Male	2.46	2.21	2.33	1.83	1.21
Female	4.62	4.73	4.87	3.84	4.74
Chick	6.71	6.93	6.13	2.98	2.9
Pullet	2.05	2.08	2.13	1.7	5.19
Layer	2.57	2.66	2.76	2.17	1.6
Cockerel	1.99	1.76	1.9	1.49	0.26
Cock	0.38	0.43	0.43	0.36	0.35
Total	**13.7**	**13.85**	**13.33**	**8.65**	**4.53**

Table 2: Monthly Average household chicken flock size dynamic.

Survival Analysis of ND in Village Chicken

A Longitudinal study was conducted to determine the Incidence rate, survival rate and predictors of ND in village chicken death during the nine months of follow-up period. Weekly and urgent telephone call visits were made during follow up period. All of the studied sick chickens reported were new cases. Of the1358 registered chicken, 202 (14.9%) survived the entire follow-up period. During the study period 843 chickens, which belonged to different age and sex categories, were found dead as a result of ND occurrence based on clinical signs. The general mortality was 62.1%. Of the 843 chicken died during the nine months interval, 85.5% (680/843) died within the third (November) and fourth (December) months of the start of follow up. The highest death 40.5% and 40.2% occurred in the December and November, respectively. The probabilities of chicken to die in these months were 44% and 29%, respectively. The 1358 chickens were followed for a total of 7448 chicken-months. The nine months (September 2014 to May 2015) incidence rate of NCD was 113.2 cases per 1000 chicken months. An overview of the duration of active surveillance and total chicken entered and lost to the follow up each month was presented in (Table 3).

Month	No. Entering Interval	No. withdrawn*	No. Exposed to Risk	Death due to ND	Probability of death in the month	Probability of Survival in the month	Cumulative Survival probability
0	1358	0	1358	0	0	1	1
1	1358	88	1314	9	0.01	0.99	0.99
2	1261	23	1249.5	32	0.03	0.97	0.97
3	1206	42	1185	339	0.29	0.71	0.69
4	825	106	772	341	0.44	0.56	0.39
5	378	0	378	0	0	1	0.39
6	378	10	373	10	0.03	0.97	0.38
7	358	25	345.5	36	0.1	0.9	0.34
8	297	19	287.5	59	0.21	0.79	0.27
9	219	12	118	17	0.14	0.86	0.23

Table 3: Kaplan Meier survival analysis of ND in village chicken.

*Withdrawn for sale, gift, slaughter, Predation

Area wise incidence of Newcastle Disease- in the case of different PAs the highest incidence of NCD was found at BiyoBisike, which is a midland PA, (141.3 cases per 1000 chicken month) while the lowest incidence was recorded at Tulu Rea, which is the highland PA, (53.4 cases per 1000 chicken month), shown in (Table 4).

Diseases	Study PA	Total chicken month	Number of Cases	Incidence rate per 1000 chicken month
ND	T/Rea	1310	70	53.4
	Biyo	1401	198	141.3
	Dibandiba	1169	140	119.8
	Tade	1400	158	112.9
	K/Fatole	1065	130	122.1
	Bika	1103	147	133.3
Total		**7448**	**843**	**113.2**

Table 4: Incidence of NCD in different PAs of the district.

Cox Proportional Hazard Analysis of NCD: Among the risk factors assessed in this study, flock size was significantly associated with NCD in chicken death ($p<0.05$). However, the risk factors like age category and sexes were not statistically significant ($P>0.05$) with NCD in village chicken death (Table 5). Village chicken kept in household flock size ≥5 to ≤9 and flock size ≥10 were significantly (log rank= 54.958, $p < 0.05$) more likely to die as compared with those chicken kept in flock size ≤4. The survival function of flock size was shown in (Figure 1).

Covariates	B	SE	Wald	HR	95% CI	df	P-value
Age ≤4month			1.325			2	0.515
≥5 to ≤ 9month	-0.085	0.091	0.863	1.0305	0.769-1.098	1	0.353
≥ 10 month	0.005	0.09	0.003	1.0618	0.843-1.197	1	0.959
Sex	-0.007	0.075	0.009	0.8798	0.857-1.150	1	0.924
Flock size ≤4			38.312			2	0
≥5 to ≤9	-0.91	0.267	11.619	1.0632	0.239-0.679	1	0.001
≥10	-0.504	0.093	29.494	1.7371	0.504-0.725	1	0.002

Table 5: Cox proportional hazards regression analysis of NCD in village chicken.

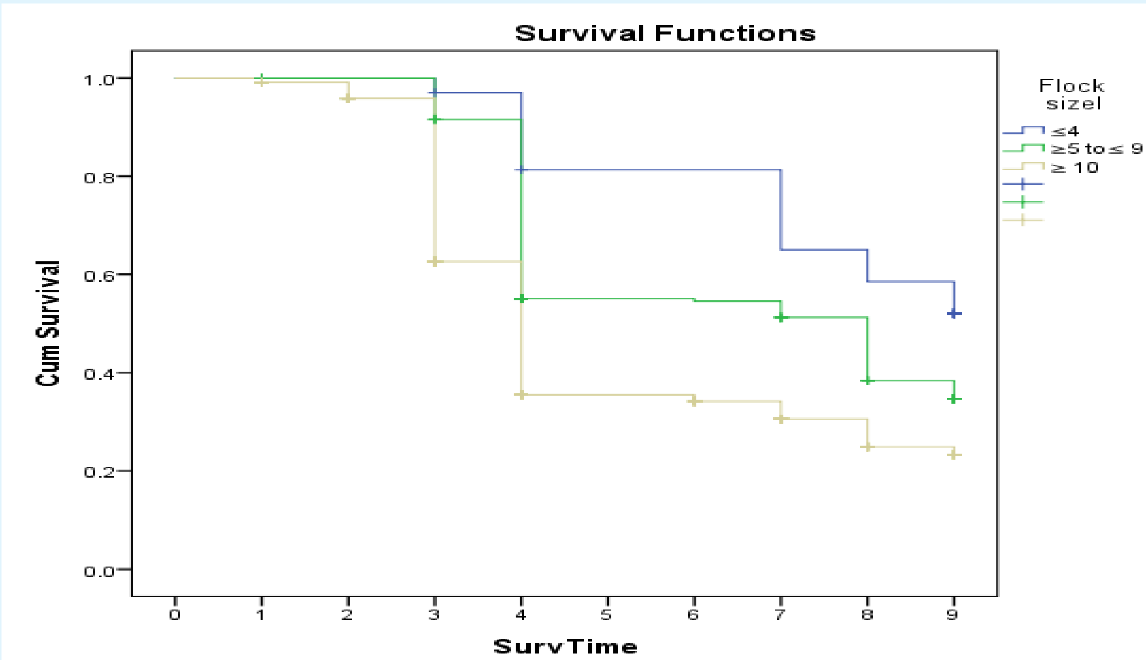

Figure 1: Survival function of ND in village chicken flock size.

Laboratory (Serology)

Over the duration of the study, serum samples of 521 chicken were collected, 242 from sick and 279 from apparently health chicken. In total 28.6% (149/521) and 20.7% (108/521) tested positive for NCD and IBD, respectively. Among the 242 sera collected from clinically diseased chicken 61.6% (149/242) and 38.4% (93/242) were positive for ND and IBD, respectively.

Seroprevalence of NCD and IBD in different PAs during disease outbreak: In the case of different PAs the highest prevalence of NCD was found at Biyo (36.2 %) while the lowest was recorded at Tulu Rea (12.5%).On the other hand the highest seroprevalence of IBD was found at Bika (24.2 %) and the lowest was recorded at Tade (18.3%). The differences in the seroprevalence of NCD among the study PAs were statistically significant ($p<0.05$) while the differences in the seroprevalence of IBD among the study PAs were not statistically significant ($p>0.05$) as shown in (Table 6).

Study PAs	Newcastle Disease				Infectious Bursal Disease			
	n	± ve	95 % CI	P-V	n	± ve	95 % CI	P-V
T/Rea	88	11(12.5%)	26.4-46.0	0.007	88	21(23.9%)	12.9 – 29.61	0.892
Biyo	94	34(36.2%)	5.5-19.45		94	20(23.3%)	14.89- 32.84	
Dibandiba	93	28(30.1%)	20.7-39.5		93	18(19.4%)	11.26- 27.45	
Tade	104	33(31.7%)	22.7-40.7		104	19(18.3%)	10.70- 25.75	
K/Fatole	80	21(26.2%)	16.5-36.0		80	15(18.8%)	10.12-27.38	
Bika	62	22(35.5%)	23.5-±47.5		62	15(24.2%)	13.42-34.97	
Total	521	149(28.6%)	24.7-32.49		521	108(20.7%)	17.24-24.22	

Table 6: Seroprevalence of NCD and IBD in different PAs of the district.

n= number sampled, ± ve= Number positive, CI= confident interval, P-V= P-value

Association of ND and IBD prevalence with Age, Sex, flock size, calendar month and agro-ecology during disease outbreak: the highest (29.6%) and the lowest (22.4%) prevalence of NCD was found in age groups of greater than or equal to 10 months and less than or equal to 4 months, respectively while, the highest (77.6%) and the lowest (8.4%) prevalence of IBD was found in age groups of less than or equal to 4 months and greater than or equal to 10 months, respectively. The differences in the prevalence of NCD among different age group were not statistically significant (p>0.05). But statistically significant (p<0.05) seroprevalence of IBD was found in different age groups as shown in (Table 7). The prevalence of NCD was found high (33.3%) in flock size level greater than or equal to ten and lower (25.8%) in flock size less than or equal to four. In the case of IBD also, higher prevalence (26.6%) in flock size greater than or equal to 10 and lower prevalence (11.3%) in the flock size less than or equal to four. The differences in the seroprevalence of NCD and IBD in relation to different flock size were statistically significant (p<0.05).Different sampling months were compared to see the variation of prevalence of NCD and IBD. Accordingly, the highest prevalence (50.8%) and the lowest prevalence (0%) of NCD were recorded in April and February, respectively while the highest prevalence (69.2%) and the lowest prevalence (10.8%) of IBD were recorded in January and March, respectively. These differences in the prevalence of NCD and IBD in relation to different sample collection months were statistically significant (p<0.05). The seroprevalence of chicken kept in lowland agro-ecology was higher (35.5%) than that of kept in highland (12.5%) in the case of NCD. The difference in the prevalence of NCD in different agro-ecology was statistically significant (p< 0.05).

Variables	Newcastle Disease				Infectious Bursal Disease			
	n	± ve	95 % CI	P-V	n	± ve	95 % CI	P-V
Sex								
Female	378	108(28.6%)	24.0-33.1	0.818	378	67(17.7%)	13.86-21.59	0.652
Male	143	41(28.7%)	21.2-36.1		143	41(28.7%)	21.22-36.13	
Age category								
≤ 4 month	49	11(22.4%)	10.6-4.2	0.588	49	38(77.6%)	65.72- 89.38	0
≥ 5 to ≤9month	175	50(28.6%)	21.8-35.3		175	45(25.7%)	19.21- 32.22	
≥ 10month	297	88(29%)	24.4-34.8		297	25(8.4%)	5.25- 11.59	
Flock size								
≤ 4	186	48(25.8%)	19.5-32.1	0.048	186	21(113%)	6.72-15.86	0
≥ 5 to ≤ 9	278	82(29.5%)	24.1-34.9		278	73(26.3%)	21.06-31.45	
≥ 10	57	19(33.3%)	21.0-457		57	14(24.6%)	13.26-35.86	
Months								
December	77	37(48.1%)	36.8-59.3	0	77	14(18.2%)	9.49 -26.87	0

January	13	3(23.1%)	0.82-47.0		13	9(69.2%)	43.06-95.41	
March	120	45(37.5%)	28.8-46.2		120	13(10.8%)	5.24-16.43	
April	118	60(50.8%)	41.8-59.9		118	28(23.7%)	16.00-31.46	
May	110	4(3.6%)	0.11-07.2		110	14(12.7%)	6.46-19.00	
Agro-ecology								
Highland	88	11(12.5%)	5.53-19.5	0.001	88	21(23.9%)	14.89-32.84	0.892
Midland	371	116(31.3%)	26.5-36.0		371	72(19.4%)	15.37- 23.45	
Lowland	62	22(35.5%)	23.5-47.5		62	15(24.2%)	13.42-34.97	

Table 7: Prevalence of ND and IBD in relation to different determinants.

n= number sampled, ± ve= Number positive, CI= confident interval, P-V= P-value

Association of risk factors with occurrence of NCD by multivariate logistic regression: Overall age category was associated with the occurrence of NCD when univariate analysis was carried out (chi square = 13.9). But up on multivariable logistic regression analysis there was no significant different in the occurrence of NCD among various age categories (Table 8). Flock size had significant effect on the seroprevalence of NCD in the study area with overall chi square of 92.46. Flocks of chicken with size greater than or equal to 10 animals per flock had an odd of having NCD that is 2.54 times higher than flocks with size less than or equal to 4 animals per flock. This difference was statistically significant. Flock size of 5 - 9 had odds of 1.14 times higher than that of those with flock size less than 4 but this difference was not significant. Months of sampling had significant effect on the prevalence of NCD in the study area. Sampling during the months of December, January, April and May gave significantly higher odds of being positive to NCD than sampling during the month of March. There was significant different in the occurrence of NCD among agro-ecology. Chicken kept in lowland and midland agro-ecology had odds of having NCD higher than chickens kept at highland.

Determinants	B	S.E.	Wald	df	Sig.	OR
Age category ≤ 4 months			3.727	2	0.155	
≥ 5 to≤9 month	-0.701	0.442	2.515	1	0.113	0.496
≥ 10 month	0.18	0.274	0.435	1	0.509	1.198
Flock size ≤ 4			9.616	2	0.008	
≥ 5 to ≤ 9	0.135	0.415	0.105	1	0.746	1.144
≥ 10	0.933	0.378	6.102	1	0.014	2.542
Sex(male)	0.066	0.272	0.059	1	0.808	1.068
Samp. Month- December			47.521	5	0	
January	3.796	0.59	41.349	1	0	44.532
March	-17.86	4184.885	0	1	0.997	0
April	2.803	0.552	25.799	1	0	16.5
May	3.259	0.548	35.407	1	0	26.02
Agro-ecology Lowland			21.86	2	0	
Midland	-2.668	0.607	19.318	1	0	0.069
Highland	-0.884	0.42	4.435	1	0.035	0.413

Table 8: Association of risk factors with NCD by multivariate logistic regression.

Association of risk factors with occurrence of IBD by multivariate logistic regression: Age category was significantly associated with the occurrence of IBD when multivariable logistic regression analysis was carried out (Table 9**).** Household flock size had significant effect on the seroprevalence of IBD in the study area. Flocks of chicken with size of 5 - 9 animals per flock had an odd of having IBD higher seropositivity than flocks with size less than or equal to 4 animals per flock. This difference was statistically significant. Flocks of chicken with size greater than or equal to 10 animals per flock had an odd of having IBD higher than that of those with flock size less than 4 but this difference was not significant. Months of sampling had also significant effect on the prevalence of IBD in the study area. Sampling during the months of December, February and March gave significantly higher odds of being positive to IBD than sampling during the months of January and April. There was no significant different in the occurrence of IBD among different agro-ecology. Chicken kept in all agro-ecology had similar odds of having IBD.

Determinants	B	S.E.	Wald	df	Sig.	OR
Age category ≤ 4 months			65.602	2	0	
≥ 5 to≤9 month	3.653	0.452	65.359	1	0	38.596
≥ 10 month	1.029	0.298	11.929	1	0.001	2.799
Flock size ≤ 4			7.855	2	0.02	
≥ 5 to ≤ 9	-1.375	0.508	7.345	1	0.007	0.253
≥ 10	-0.539	0.425	1.611	1	0.204	0.583
Sex(male)	-0.358	0.287	1.553	1	0.213	0.699
Cal. Month- December			25.579	5	0	
January	-0.578	0.535	1.165	1	0.28	0.561
February	2.188	0.812	7.257	1	0.007	8.916
March	1.13	0.466	5.872	1	0.015	3.097
April	-0.608	0.497	1.499	1	0.221	0.544
May	0.658	0.418	2.477	1	0.115	1.932
Agro-ecology Highland			1.745	2	0.418	
Midland	-0.685	0.57	1.441	1	0.23	0.504
Lowland	-0.614	0.485	1.6	1	0.206	0.541
Constant	-1.049	0.774	1.835	1	0.176	0.35

Table 9: Association of risk factors with IBD by multivariate logistic regression.

Discussion

An average household chicken flock size was found decreased in December and January during the study period. Except male chicken which suffered from population reduction in different months, the female chicken population reduced in December during the follow up period. Similarly, high population reduction of chick was also seen in December and January. The chicken population reduction during December and January was due to NCD outbreak occurred during this time. This result is in agreement with the finding of Chaka et al. [10] that reported households were found their flock size reduced mainly due to diseases during dry season. On the other hand, respondents indicated that the decreased flock size was due to diseases, predation and unknown case. This finding is in agreement with Chaka et al. [16] and Nega et al. [17] who reported respondent's indication that their flock size decreased due to diseases. These indicated that households had lost their chickens, possibly due to incidence of diseases in their flocks, among other factors. Chicken off take due to sell following occurrence of diseases outbreak, slaughter and gift also contributed to reduce flock size. The majority of the respondents indicated ND locally known as "Fengel" was the leading disease to cause mortality of chicken in the village. This was corroborated, in many cases, by the farmer's report of frequent diseases symptom in their flocks, and sero-positive during the sampling period. Different authors; Nega et al. [17] and Selam et al. [8] and Chaka et al. [16]

also reported the indication of respondents that diseases, mostly ND, were the important causes for chicken mortality in village. But, in contrary with the current study, Selam et al. [8] and Nega et al. [17] reported, respondents indicated predator was the major cause for chicken loss in the village. In this study low percent of respondent indicated predation was a major cause of village chicken loss. This difference could be due to increased awareness of farmers to use different techniques that reduced exposure of chicken to predation. This result agrees with the findings of Tadelle and Ogle [18] who reported disease as the most important factor in the death of chicks. On the other hand respondents indicated disease occurrence, specifically NCD, was higher at dry season. This result disagrees with the findings of Selam et al. [8] who reported, the respondents indicated disease occurrence was higher at short and long rainy season. But it is supported by Chaka et al. [16, 10], Nega et al. [17] and Zeleke et al. [13] who identified human activity and increased in the chicken market turnover during dry season could leads to outbreaks of chicken diseases particularly NCD have been attributed to high prevalence during dry season. In the current study area, the villagers recognize the season when diseases will occur and they dispose of their chickens by sale, thus initiating or sustaining outbreaks.

Longitudinal study was conducted to determine the incidence rate and predictors of NCD in village chicken death during the nine months of follow-up period. During the study period birds belonged to different age and sex categories, were found dead as a result of NCD occurrence. High mortality was recorded within the third (November) and the fourth (December) months of the observation period. During these months, human activity and chicken market turnover was high because of holyday known as ''X-mass''. The general mortality rate that comparable with the study conducted by Mohammed et al. [14] From North Western Amhara, Biswas et al. [19] and Barmon et al. [20] from Bangladesh was recorded. The mortality rate reported by Biswas et al. [19] was lower than the present finding. This could be due to different climatic condition which was favorable for the transmission and occurrence of NCD in Ethiopia than Bangladesh.

Serological study was conducted to evaluate the prevalence of NCD and IBD in active clinical case and apparently health chicken during disease outbreak. Over the duration of the study, the recorded seroprevalence of NCD is in concurrence with Zeleke et al. [13] and Tadesse, [9] who reported seroprevalence of 19.78% and 32.2%, from Southern and Rift valley districts of Ethiopia and central Ethiopia, respectively. Similarly, Chaka et al [10] reported prevalence of 21.5% and 34.5% from Adami Tulu Gido Kombolch and Ade'a wereda, respectively. Serkalem et al. [11] also reported prevalence of 28.57%, 29.69% and 38.33% from Debreberhan, Sebeta and Nazaret, respectively. This study showed NCD is one of the major infectious diseases that reduces the number and productivity of traditionally managed chickens in the study area. The data clearly indicate that local chickens kept under free-range traditional management systems in which chickens literally scavenge their own feed and water were easily exposed to NCD virus from the simply throw away of dead body of the birds in the field that might create a good ground for disease transmission.

The prevalence of IBD in this study in agreement with Mazengia et al. [21] and Mazengia et al [22] who reported seroprevalence of 17.4% and 29.4% from Farta and Bahirdar, respectively. But the seroprevalence of IBD found in this study was higher than Bettridge et al. [23] who reported 3.6% prevalence from Horro and Jarso [24] who reported 7% prevalence from Andasa poultry farm. While, it was found lower than Shiferaw et al. [12] overall report of 83.1% prevalence from eight districts of Ethiopia, Hailu et al. [25] reports of 76.64% seroprevalence from three districts of West and South West Showa, Tesfaheywet and Getnet [26] reports of 82.2% from Central Ethiopia. The variation in reports of IBD seroprevalence by different author in different area could be related with the dissemination of IBD through distribution of improved breed of chickens from infected poultry breeding and multiplication centers to the village chick. Related to the above rationale, lower prevalence of IBD recorded in the current study area was because, most of the households decline to rear the improved chicken breeds, and rather they entirely depended on the indigenous local breeds. Shiferaw et al. [12] reported the highest seroprevalence of IBD in cross breed of chicken and the lowest in indigenous local breed of chicken.

In this study relatively higher prevalence of NCD and IBD was recorded in male chickens than female, however the difference was not statistically significant ($P>0.05$). This finding was similar with that of Serkalem et al. [11], Zeleke et al. [13], Shiferaw et al, [12], Kassaa and Molla, [27] and Tesfaheywet and Getnet, [26] who reported the absence of influence of sex on the prevalence of ND and IBD. The seroprevalence of IBD was found high in age group ≤4 months, while the lowest prevalence was recorded in age groups ≥10 months. Statistically significant difference ($p<0.05$) was observed in the seroprevalence between different age groups. A comparable seroprevalence of IBD in young chicken was reported by Shiferaw et al. [12] and Hailu et al. [25]. Singh and Dhawedkar [28] reported that the prevalence was highest (61.82 %) in chickens between 7 and11 weeks old and lowest in those above 22 weeks of age. The susceptibility of chickens to IBDV is influenced by their age. The maximum susceptibility was observed between 2 and 7 weeks of age [29].

A statistical significant difference prevalence of NCD and IBD was observed in different chicken flock size. The highest seroprevalence of NCD and IBD were found in chicken flock size ≥10 and the lowest prevalence of NCD and IBD were found in chicken flock size ≤4. This difference might be due to the fact that increased chicken population number is a factor for the transmission and widely occurring of the diseases.

Different sampling months were compared to see the variation in prevalence of NCD and IBD. Statistical significant difference prevalence was found; the highest and the lowest seroprevalence of NCD were recorded in April and February, respectively while, the highest and the lowest prevalence of IBD were recorded in January and March, respectively. This was substantiated with the farmer's report of high diseases occurrence in the months when Ethiopian holydays celebrated (Easter in April and X-mass at the end of December). Because, during this period diseased chickens were brought from different areas and sold by traders, that could facilitated transmission and widely occurrence of diseases during this months.

The seropositivity of chicken kept in lowland agro-ecology was higher than that of kept in highland in the case of NCD. This difference in the prevalence of NCD in different agro-ecology was statistically significant ($p < 0.05$) but no statistical significant was seen in different agro-ecology in the case of IBD. This records was agree with findings of Zeleket al. [13], Tadesse [9] and Belayheh et al. [30] who reported a higher prevalence of NCD in the lowland than highland. Serkalem et al. [11] reported comparable results indicated, although there was no statistically significant difference between different agricultural-climatic zones in NCD virus seroprevalence rates, a relatively higher seroprevalence was observed in Lowland followed by midland than in Highland. According to Zeleke et al. [13] and Serkalem et al. [11], the possible reason for this could be there are few chickens in the highland area of the country and chicken population number is a factor for the transmission of the disease. Another explanation may also be because of ecological variations in NCD activity and may perhaps be a reflection of the impact of environment on the speed of transmission and viability of NDV and epidemiology.

Conclusion and Recommendation

A prospective study coupled with seroepidemiology could be a useful tool to assess the status of major village chicken diseases in an area and provide insight for further investigations. This study results showed that the village chicken population is endemically infected with NDV and IBD, with a high proportion of household flocks experiencing new cases. The data clearly indicated that, local chickens kept under free-range traditional management systems in which chickens literally scavenge their own feed and water in the six PAs were exposed to NCD and IBD. Massive mortality in November and December during the follow up and higher infection rate in February and April from serology indicated that there is a tendency towards higher incidence and periodic outbreaks of the disease in different seasons. NDV in household chickens pose a significant threat to the development of traditional poultry production sector in Ethiopia and IBD is also appearing as a significant threat. Based on the above conclusion,

The following recommendations were forwarded:

- Improvement of village chicken production and management which is at least partly has a role on successful control of these diseases.
- Programmed vaccination at the household level could be considered to reduce the seasonal incidence and mortality of both diseases.
- Further study is warranted to better understand to characterize virus strains circulating in the study area in order to properly aid control of ND and IBD.
- Further study is necessary to understand the interactions of these infectious poultry diseases and to estimate their impact on the backyard poultry production system.

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