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Newcastle disease: Seroprevalence and associated risk factors in backyard and small scale chicken producer farms in Agarfa and Sinana Districts of Bale Zone, Ethiopia

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A cross-sectional study on seroprevalence of Newcastle disease virus (NDV) antibodies in backyard and small-scale chicken producer farms in Agarfa and Sinana districts was conducted using hemagglutination inhibition test (HAI) from February, 2015 to May, 2015. A total of 384 chicken sera were randomly collected from ten kebeles of the selected districts. Hemagglutination inhibition (HAI) test was used to analyze 384 chicken sera for NDV antibodies and the overall seroprevalence rate of 27.86% was found. A higher seroprevalence of 33.04% was observed in Sinana district when compared to Agarfa (20.13%) district. The prevalence in each kebele ranges from 15.63% to 40%; the highest prevalence of 40% was found at Horaboka, but insignificantly associated with Newcastle disease (ND) seropositivity. A Chi-square computed statistical analysis indicated that origin ($\chi 2=7.6526$; $p<0.006$), sex ($\chi 2=6.9134$; $p<0.009$) and type of chicken (layers/broilers) ($\chi 2=11.2443$; $p<0.001$) were the major risk factors for ND infection in the studied areas. The difference, however, was not statistically significant ($p>0.05$) for age (adult/young), breed (exotic/cross/indigenous (local)), contact with other flocks, access to feed and water, and seasonal occurrence. Multivariable logistic regression statistical analysis revealed that origin and type (layers/broilers) were significantly associated with ND seropositivity ($p<0.05$). Consequently, origin was statistically identified to be the major risk factor for ND to occur in relation to other factors (Adjusted Odds Ratio (AOR) =2.12). The study showed that majority of the chicken population in the studied area was susceptible to the pathogenic NDV infection. Therefore, more proactive measures should be taken to protect the chicken population from ND infection to reduce its economic impact to the poultry industry.

Key words: Agarfa, chicken, Newcastle disease, risk factors, seroprevalence, Sinana.

INTRODUCTION

Poultry production plays a major role in the economy particularly of developing countries (Mazengia, 2012).

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The larger proportion of rural poultry in the national flock population of developing countries makes them worth paying attention to improved management and breeding. At national level in Ethiopia, 99% of the total, 56.5 million, estimated chickens are contributed by village poultry production while only 1% is from intensive exotic breed maintained under intensive management system (Tadelle and Ogle, 2001; Dinka et al., 2010).

In village systems, farmers keep poultry for diverse objectives. They are raised for purposes of hatching, sale, home consumption, sacrifices (healing ceremonies) and gifts (Mazengia, 2012). In Ethiopia, village chickens have been reared for a long time for similar purposes. Constraints which restrict the potential of village chickens in Ethiopia include; low inputs of feeding, poor management, the presence of diseases of various natures and lack of appropriate selection and breeding practices (Ashenafi, 2000; Tadelle and Ogle, 2001).

Among the constraints, poultry diseases are considered to be the most important factor responsible for reducing both the number and productivity of chickens (Tadesse et al., 2005). A growing concern reveals that as there is introduction of diseases of various etiologies into several poultry farms concurrent with importation of exotic breeds to backyard chickens. Furthermore, intensification is aggravating the rapid spread of the prevailing infectious diseases between and within poultry farms. And the distribution of these exotic breeds to farmers is creating a great threat to the indigenous backyard chickens (Zeleke et al., 2005a). Among these threats, viral diseases like Newcastle disease (ND) is the major health constraints inflicting heavy losses (Tadelle and Ogle, 2001; Zeleke et al., 2005a, b).

Newcastle disease (ND) is one of the most important viral diseases (Orsi et al., 2010). It is an acute infectious viral disease of domestic poultry and other species of birds regardless of variation in sex and age (Haque et al, 2010). The disease is characterized by respiratory, nervous system impairment, gastrointestinal and reproductive problems (Tiwari et al., 2004).

Sources of infection for NDV are exhaled air from infected birds and contaminated feed and water and transmission is mostly 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. Much of the spread of ND in village is probably via human agents (Ashraf and Shah, 2014). An outbreak of ND is unpredictable and discourage villager from paying proper attention to the husbandry and welfare of their chickens (Spradbrow, 2001).

Various studies have been conducted to determine the epidemiology of ND in various countries in Africa. In study conducted in Ethiopia by Tadesse et al. (2005) and Ashenafi (2000), the seroprevalence rates of 28.57, 29.69, 38.33 and 43.68% were found in Debre Berhan, Sebeta, Adama and Central Ethiopia (among local scavenging chickens kept under a traditional management system), respectively. Another study conducted in two districts of Eastern Shewa Zone, Ethiopia by Chaka et al. (2012) to estimate the seroprevalence of ND (and other poultry diseases being not considered in this study) in the wet and dry seasons and they reported the overall seroprevalence of ND was 5.9% during the dry season and 6.0% during the wet season.

In general, the epidemiology of ND in village poultry in Ethiopia is poorly understood and there is no appropriate investigation and control strategy designed against the disease. This is due to lack of disease monitoring capacity in the Veterinary Services Department of the Ministry of Agriculture and Rural Development (Tadelle and Jobre, 2004). Farmers start to consider, therefore, losses due to diseases as normal and natural (Tadelle, 1996; Nasser, 1998) and they fail to report outbreaks to the veterinary authorities.

Though all the above study reveals that as ND seriously devastating poultry industry in Ethiopia, there is no published data (information) about the seroprevalence of this disease in poultry industry threat in Bale Zone in general and in Agarfa and Sinana districts in particular. This paucity of information on the presence and seroprevalence of ND in backyard and small scale poultry producer farms may reflect a lack of resources for disease surveillance and control in poultry production system.

In addition, the diagnostic coverage of poultry diseases in Ethiopia is limited to the extent that, even from commercial farms, only a few cases are brought to National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta or the National Veterinary Institute (NVI), Bishoftu. Most poultry disease outbreaks, particularly in more remote parts of the country, remain undiagnosed and dead chickens are simply discarded (Chaka et al., 2012). Therefore, information on the seroprevalence and significance of ND can only readily be obtained through serological studies on apparently healthy and unvaccinated chickens.

Hence, this study was conducted to determine the seroprevalence of ND that potentially affect backyard and small-scale poultry producer farms in Sinana and Agarfa districts of Bale Zone, and to assess the risk factors contributing to ND seropositivity in the districts. Therefore, our study could complement the paucity of information about seroprevalence of ND and associated risk factors in poultry industry sector of the study areas.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Sinana and Agarfa districts of Bale zone, Oromia Regional State, South East of Ethiopia. Sinana district

is located at 430 km southeast of Addis Ababa. The area is situated at 7° 7' N and 40° 10' E and 2400 masl. The mean average rainfall of the area is 353 mm. For the same period, average annual maximum temperature is 21.2°C and minimum temperature is 9.4°C. The dominant soil type is pellic vertisol and slightly acidic (pH=6). Agricultural production system of the study area is mixed farming. There are about 287,825 bovine, 47,121 ovine, 10,600 caprine, 26,020 equine and 60,000 poultry are found in Sinana district (SDAO, 2014).

Agarfa district is located at 464 kms south east of Addis Ababa. The area is situated at 6°11' N and 40°3' E and 2350 masl. The mean average rainfall of the area is 880 mm and bimodal. The average annual maximum temperature is 24.75^{o}C and minimum temperature is 7.1^{o}C. The dominant soil type is clay soil and slightly acidic (pH = 5.8). Agricultural production system of the study area is mixed farming. There are about 229,206bovine, 63,485 ovine, 15,674 caprine, 33,777 equines and 40,150 poultry in Agarfa district (ADAO, 2014).

Sampling method and determination of sample size

The sample size was calculated according to Thrusfield (2007) by considering 50% expected prevalence (P) (since there was no reasonable research done in these districts so far), 95% confidence interval (CI) (Z=1.96) with 5% desired absolute precision(d), using the formula N= (Z) 2 P (1-P)/d^{2}for simple random sampling. The calculated requiredsample size (N) was 384.

Accordingly; the total numbers of sample required for this study was 384 chickens from both backyard and small scale poultry producers.

Sinana district contains 20 kebeles while Agarfa district contains 19 kebeles. Five kebeles from each district were selected purposely by their proximity to roads, accessibility of infrastructure and poultry holdings of each kebele. Prior to commencement of the study, list of all households (HHs) of those kebeles (sampling frame) was obtained from both district Agricultural Office.

Inclusion criteria: Apparently healthy chickens with history of no vaccination were included.
Exclusion criteria: Apparently healthy chickens with history of vaccination were excluded.

Study population

The study population was all apparently healthy chickens with history of no vaccination in the selected districts. According to districts agricultural office, there were about 60,000 and 40,150 poultry flock in Sinana and Agarfa districts (SDAO, 2014; ADAO, 2014), respectively. The studied animals were consisting of 384 apparently healthy chickens with history of no vaccination. The chickens sampled were selected by simple random sampling method from backyard and small scale poultry producer farms.

Study design

A cross-sectional type of study supported by questionnaire survey was conducted to determine the seroprevalence of ND and its associated risk factors in backyard and small scale producer farms in the two selected districts. Questionnaire survey was conducted to have a birds-eye-view of poultry diseases in the afore-mentioned districts. In the two selected districts poultry owners were interviewed with semi-structured questionnaire. Emphasis was given on the frequent clinical symptoms manifested whenever outbreaks of poultry diseases occurred in the respective study sites. Tentative diagnosis was made based on the classical disease manifestation and vaccine was recommended for healthy chickens accordingly. The questionnaires was prepared, pre-tested and adjusted by translating in to local language (Afan Oromo) and administered by the interviewer. The questionnaire was focused on the potential risk factors and was conducted after carefully explaining the purpose of the work to the interviewees.

Sera collection and testing

Sera collection

After plucking few feathers from the ventral surface of the humeral region of the wing and wiping the site with cotton damped with alcohol, approximately 4-5 ml blood samples were collected from the brachial vein, using plain vacutainer and with 18-20 gauge hypodermic needles. The vacutainer tubes were labeled and set tilted on a table overnight at room temperature to allow clotting. Then sera was filled into storage vials (cryovials) with appropriate identification and stored at –20ºC until transported to NVI and the HAI was performed.

Haemagglutination-inhibition test (HAI)

HAI test was conducted according to the procedures of Beard and Wilkes (1985) and OIE (2002). The test was undertaken at NVI, Bishoftu, Ethiopia, by running two fold dilutions of equal volumes (0.025 ml) of phosphate buffered saline (PBS) and test serum (0.025 ml) in a U bottomed micro titer plates. Four haemagglutinating units (HAU) of virus/antigen were added to each well and the plate was left at room temperature for a minimum of 30 min. Finally 0.025 ml of 1% (v/v) chicken red blood cells (RBCs) was added to each well and, after gentle mixing, the RBCs were allowed to settle for about 40 min at room temperature. The HAI 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 RBCs stream at the same rate as the control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) were considered to show inhibition after greater than or equal to 4(logarithm to base 2) was taken as positive.

Data storage and analysis

Data generated from questionnaire survey and laboratory investigations were recorded and coded using Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA). The seroprevalence was calculated as the number of seropositive samples divided by the total number of samples tested. To identify association of seropositivity with the potential risk factors (origin, sex, age, breed (indigenous/cross/exotic), type (layers/broilers), contact with other flock, seasonal occurrence and access to feed and water were computed by Pearson's Chi-square and multivariable logistic regression tests. A *p*-value <0.05 was considered statistically significant.

RESULTS

Overall seroprevalence of Newcastle disease in the studied districts

In the present study, an overall seroprevalence of

Table 1. Overall seroprevalence of HAI test result of ND in backyard and small scale poultry production system of the study districts.

Haemagglutination inhibition test result (HAI)	Selected districts		Total (N)	Overall prevalence (%)
	Agarfa N (%)	Sinana N (%)		
Positive	31(20.13)	76(33.04)	107	27.86
Negative	123(79.87)	154(66.96)	277	72.14
Total	154(100)	230(100)	384	100

Pearson χ^2 (1) = 7.6526; Pr = 0.006; N: Number of chickens tested.

Table 2. Seroprevalence of NDV antibodies in different selected kebeles of the selected districts.

Selected kebeles from the two districts	Positive samples (N)	Negative samples (N)	Total	Prevalence (%)
Agarfa				
Ali	11	35	46	23.91
Amigna	6	16	22	27.27
Anbentu	5	27	32	15.63
Elebidu	4	20	24	16.67
Ilani	5	25	30	16.67
Sinana				
Besaso	13	40	53	24.53
Horaboka	24	36	60	40
NanoRobe	13	23	36	36.11
Shallo	7	25	32	21.88
Shaya	19	30	49	38.78

Pearson χ^2 (9) = 15.4957; Pr = 0.078; Pr=Precision value.

27.86% was estimated by HAI test. A higher seroprevalence of 33.04% was observed in Sinana when compared to Agarfa (20.13%) as depicted in Table 1.

Seroprevalence of NDV antibodies in selected kebeles of the study districts

Of 10 kebeles selected, Horaboka was with the highest ND seroprevalence (40%) while Anbentu was the least (15.63%). There was no significant association between the selected kebeles of the studied districts and ND seropositivity (Table 2).

Chi-square analysis of association of the putative risk factors with ND seropositivity

A Chi-square analysis revealed that origin, sex, and type of chickens were significantly associated (p<0.05) with ND seropositivity among other factors considered during the study (Table 3).

Multivariable logistic regression analysis of putative risk factors associated with ND seropositivity

The logistic regression analysis of the putative risk factors indicated that chickens originated from Sinana were more likely to be infected (AOR= 2.12, 95 % CI: 1.30-3.46) with ND than chickens from Agarfa (Table 4).

DISCUSSION

The present serological study revealed that the presence of circulating antibodies of ND among chickens sampled from backyard and small scale poultry producer farms of Agarfa and Sinana districts of Bale Zone. An overall seroprevalence of 27.86% was obtained using HAI (≥4log2) from the two districts. This finding is comparable to 31.2% of anti-NDV antibodies observed by Salihu et al. (2012) in Nassarawa State, 23.6% by Abraham et al. (2014) in Delta State of Nigeria and 32.2% by Tadesse et al. (2005) in central Ethiopia, but the result of the present study is considerably higher than previous report by Zeleke et al. (2005b), Regasa et al. (2007) and Chaka et al. (2012), who reported seroprevalences of 19.8% in the southern and Rift Valley districts, 11% in southern Ethiopia and 6% in Eastern Shewa zone, respectively. However, our result is lower than the prevalence of 43.68% reported by Ashenafi (2000) in central Ethiopia among local scavenging chickens kept under a traditional management system, 46% in village chickens in Borno State (EL-Yuguda et al., 2007) and 54.67% in Nasarawa

Table 3. Chi-square analysis of association of the putative risk factors with ND seropositivity.

Variable		Number tested	Number positive N (%)	χ^2 (*p*-value)
Origin	Agarfa	154	31(20.13%)	7.6526 (0.006*)
	Sinana	230	76(33.04%)	
Age	Adult(>6mos)	219	62(28.31%)	0.0504(0.822)
	Young(3-6 mos)	165	45(27.27 %)	
Sex	Female	321	98(30.53%)	6.9134 (0.009*)
	Male	63	9(14.29%)	
Breed	Cross	197	57(28.93 %)	0.7940 (0.672)
	Exotic	45	14 (31.11%)	
	Indigenous	142	36(25.35%)	
Type	Broilers	74	9(12.16 %)	11.2443(0.001**)
	Layers	310	98(31.61 %)	
Contact with other flock	Yes	170	40(23.53%)	1.7423 (0.187)
	No	214	67(31.31%)	
Access to feed and water	Roaming	218	55(25.23%)	1.7423(0.187)
	Confined	166	52(31.33%)	
Seasonal occurrence	At the beginning of rainy season	306	85(27.78%)	0.0056(0.940)
	At the end of rainy season	-	-	
	Throughout the year	78	22(28.21%)	

Mos; months; *Statistically significant; **Highly statistically significant.

Table 4. Multivariable logistic regression analysis of putative risk factors associated with ND seropositivity.

Variable	ND test result		Odds ratio		*p*-value
	Negative	Positive	COR (95%CI)	AOR (95%CI)	
Origin					
Sinana	154(67.0)	76(33.0)	1.96(1.21, 3.20)	2.12(1.30, 3.46)	0.003
Agarfa	123(79.9)	31(20.1)	1	1	
Sex					
Female	223(69.7)	97(30.3)	2.35(1.15, 4.81)	0.18(0.02, 1.71)	0.136
Male	54(84.4)	10(15.6)	1	1	
Type					
Layers	213(68.5)	98(31.5)	0.31(0.15, 0.64)	0.06(0.01, 0.60)	0.016
Broilers	64(87.7)	9(12.3)	1	1	

AOR, Adjusted Odds Ratio; COR, Crude Odds Ratio; CI, Confidence Interval; 1, Reference.

State (Salihu et al., 2012).

This could be explained by differences in study settings or by exposure to mild virus strains that induced immunity but did not kill many chickens. The presence of lentogenic, or possibly mesogenic, NDV in backyard/ small scale chicken producing farms in an area may result in a constant cycle of infection that periodically boosts the immunity of all exposed chickens, resulting in a higher proportion of chickens with antibodies (Martin, 1992; Chaka et al., 2012). Another reason for variation

between studies could be subjectivity and variation in HAI cutoff values used for the interpretation of the result. For instance, some authors considered an HAI titer ≥ 1log2 as positive (Bouzari and Mousavi, 2006; Biswas et al., 2009), whereas others used cut-off titers of ≥3log2 (Tadesse et al., 2005; Zeleke et al., 2005b). However, the present study used ≥4log2 which is similar with the cutoff values used by Gutierrez-Ruiz et al. (2000).

None of the chickens sampled had a history of previous vaccination against ND. It is therefore deduced that antibodies detected in the back yard and small scale chicken producing farms in this study was as a result of natural infection by NDV. Therefore, the 27.86% seroprevalence rate of ND antibodies in the two districts could be attributed to factors such as the management system in traditional production which may serve as a stress factor and favour infection.

Also, the continued exposure to array of infectious agents and wild birds, nutritional deficiencies, the absence of disease control through vaccination, contact of birds of one rural area with those of another rural area through gift and sale of rural chickens which in some cases are diseased or carriers of some diseases may facilitate the spread of diseases like ND among flocks (Musa et al., 2009).

The present study revealed that the origin of the chickens was significantly associated with ND seropositivity (p<0.05) and it was also statistically identified that origin was the major risk factor for ND seropositivity to occur in relation to other factors within the same agro-ecology. The results showed higher individual chicken seroprevalence in Sinana (33.04%) when compared to Agarfa (20.13%). According to districts agricultural office, there were about 60,000 and 40,150 chicken flock in Sinana and Agarfa districts (SDAO, 2014; ADAO, 2014), respectively. Therefore, the higher prevalence recorded in Sinana district can be attributed to more chicken had been sampled (230 chicken sampled) compared to Agarfa (154 chicken sampled) district. Zeleke et al. (2005b) and Tadesse et al. (2005) reported low altitudes do have higher seroprevalence than the high altitude in their studyand they were investigated as there were few chickens in the highland area and chicken population number is a factor for the transmission of the disease in their study. Contrary to these findings, the present study investigated significant variation within the almost closer agro-ecology (Sinana 2400 and Agarfa 2350 masl) (variation within higher altitude).

The difference in the seroprevalence between adult (> 6months) and young (3-6 months) of age was statistically insignificant (p>0.05), which disagrees with the finding of Vui et al. (2002) which stated that the young (3- 6 months-old groups) had a significantly lower NDV antibody titre than the adult (> 6 month-old age groups). This can be hypothesized to be due to more frequent exposure of older birds to field virus, which might have survived the disease at an earlier age (Getachew et al., 2014).

This study also revealed a higher seroprevalence rate among the female (30.53%) compared to male chickens (14.29%) with statistically significance difference (p <0.05). Our finding corroborates the findings of Tadesse et al. (2005), who reported a slightly higher prevalence of 32.63% among female chickens when compared with a prevalence of 31.63% among male chickens in Ethiopia.

In contrary to this finding, a study conducted by Zeleke et al. (2005b) in the Southern and Rift Valley districts of Ethiopia, ND shows a higher prevalence rate among males (21.74%) than among females (19.16%).

The highest seroprevalence was observed in exotic breed than in the indigenous (local) and cross-bred chicken in the present study. The difference, however, was not statistically significant. An insignificant difference (p>0.05) in the seroprevalence between the indigenous (local) and cross breeds of chickens (excluding exotic breed) was reported by Vui et al. (2002) which is consistent with the present findings. In contrast to this, the relatively higher overall seroprevalence rate of ND virus antibodies in local chickens reported by Tadesse et al. (2005) attributed to a number of factors. However, the exotic breed sampled in this study was lower than the indigenous (local) and cross-bred which results in difficulty of interpretation of our findings because the question of breed susceptibility to ND is still controversial (Awan et al., 1994). Hence, this area needs an indebt study to unveil the factors responsible for this difference.

There was statistically significant association (p<0.05) between type of the chickens (layers or broilers and seropositivity of ND in the present study. A higher prevalence recorded in layers than in broilers chicken can be attributed to more layers been sampled. Khan et al. (2011) reported that relatively high level of antibodies against ND in unvaccinated birds observed during the study, (33% in egglaying hens) indicated a high prevalence of NDV infections in village chickens. The birds showing detectable levels of antibodies were considered exposed, while those having undetectable level of antibody titer against ND were considered as non-vaccinated.

The issue of seasonal ND peaks has always been controversial and may vary according to the environmental, nutritional and socio-economic conditions, under which poultry is kept (Vui et al., 2002).There was no observed seasonal variation in seroprevalence in the present study, suggesting that the disease is widespread and occurs throughout the year in the studied area which is consistent with the report of Chaka et al. (2012). However, in contrary to this finding, Awan et al. (1994) reviewed the literature and found reports of ND peaks during (Asadullah, 1992; George, 1991; Mishra, 1992) and at the end of the dry season while Nguyen (1992) reported that in Viet Nam the ND peaks generally occur at the beginning of the rainy season (September-March)

and Martin (1992) in a review concluded that ND outbreaks are often associated with the change of seasons, specifically at the start of the wet season.

Conclusion

This study established that ND is endemic in Agarfa and Sinana districts of Bale zone. Higher seroprevalence was observed in Sinana when compared to Agarfa with significance difference. Origin was statistically identified as the major risk factor for ND seropositivity to occur in relation to other factors while age, breed (exotic/cross/indigenous), contact with other flock, seasonal occurrence, and access to feed and water were insignificantly associated with ND seropositivity. This finding, apart from being of economic significance, it is also of nutritional importance because of the high mortality of the birds, which calls for adoption of preventive measures to help curb the devastating effects of the NDV. The prevailing ND sero positivity in the chicken production system indicates the importance of ND in poultry industry of the studied areas and therefore, to effectively control ND, more attention should be given to those areas by adopting prophylaxis through the use of heat resistant ND vaccines for the chickens.

Conflict of Interests

The authors have not declared any conflict of interest.

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