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Seroprevalence of Newcastle Disease Virus Antibodies in Village Chickens in Kersana-kondalaity District, Ethiopia

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Abstract: A cross-sectional study on sero-prevalence of Newcastle disease virus (NDV) antibodies in village chickens in Kersana-kondalaity district was conducted using Blocking-Enzyme Linked Immunosorbent Assay (b-ELISA) from January to March 2009. A total of 355 chicken sera were randomly collected from eight peasant associations (PA). An overall sero-prevalence of 5.6% (3.2-8.0% at 95 % CI) was found and five (62.5%) of the eight PA sampled had chickens that were positive for antibodies against NDV. The prevalence in each PA ranges from 0% to 28.1% and the highest prevalence (28.1%) was found at Harbu PA, located just near to market area. The prevalences of chicken's serum antibody in the highland and lowland area were 0.9% and 7.8% respectively. Statistically significant ($p<0.05$) difference in prevalence of Newcastle disease virus antibodies was found between highland and lowland, local and cross breeds and young and adult chickens. The difference, however, was not statistically significant ($p>0.005$) for male and female. The study showed that majority of the chicken population in the study area were susceptible to the pathogenic NDV infection. Thus, routine vaccination program is recommended.

Key words: Newcastle Disease Virus Antibody · Seroprevalence · Village Chicken · B-Elisa

INTRODUCTION

Poultry industry in Ethiopia is dominated by the traditional sector. Free-range poultry keeping is most common in the country. The chickens reared under traditional or "backyard" conditions accounts for 99%, while only 1% chickens are kept under intensive management system in commercial farms [1]. Diseases and especially the devastating Newcastle disease (ND) are perceived to be the main constraint [2-4], which frustrates any investment in this system in the country. Thus, the potential of the free-range chicken production has not been exploited. Therefore, if any success is to be achieved in improvements for free-range village chickens production, it will inevitably depend on the successful control of major poultry diseases in general and ND in particular.

For this to be feasible a base line date should be established to assist in formulating ND control programmes. Consequently, epidemiological studies on free-range village poultry have to be carried out in Ethiopia to generate data, which could be used in the formulation of Newcastle disease control program. Nevertheless, there has been limited information available on prevalence of ND in different parts of the country. Therefore the objective of this paper was to determine prevalence of ND virus antibodies in village chickens and identify possible risk factors in the study area

MATERIALS AND METHODS

Study Area and Population: The study was conducted in Kersana Kondalaiti district, Ethiopia. The altitude of this district ranges from 1400 to 3000 meters above sea level. This area experiences a binominal rainfall pattern with a long rainy season from June to September and short rainy season from March to April. There was thirty two PAs in the district.

The most recent estimate of chicken population in the district at the time of study was 64,395 [1]. Most of the

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chickens were local breeds and the rest were crosses. There was no history of vaccination program in the district at the time sampling.

Sera Collection: Approximately 2 ml of blood was collected from the brachial vein of chickens using a 3ml syringe and a 23-gauge needle after disinfecting the site with cotton soaked in 70% ethanol. The whole blood collected from local chickens was labeled and allowed to clot under normal atmospheric condition within the syringe. Then the clear serum was harvested into labeled cryovials and stored at -20°C until the b-ELISA test was carried out. A total of 355 sera samples were collected from eight PAs randomly.

Blocking - Enzyme Linked Immunosorbent Assay: The SVANOVIR® Newcastle disease antibodies Blocking-ELISA kit was used to detect NDV specific antibodies in the serum samples. The test was performed as described previously [14] and according to manufacturer's recommendations (Svanova Biotech AB, Uppsala, Sweden). Briefly, 50 µl of PBS -Tween Buffer and 50 µl of undiluted serum were added to each of the selected non infectious NDV antigen coated wells and mix thoroughly. Then the plate were sealed and incubated for 30 minutes at room temperature (around 25 °C) before rinsing the plate three times with PBS -Tween Buffer. Then 100 µl horseradish peroxidase (HRP) conjugated monoclonal antibodies directed to NDV were added to each well and the plates were again sealed and incubated for 30 minutes at room temperature. After that 100µl substrate solution were added to each well and incubated for 10 minutes at room temperature after rinsing the plate three times with PBS –Tween Buffer. The reaction was stopped by adding 50 µl stop solution to each well and mixed thoroughly. Finally, the optical density (OD) of the controls and samples were measured at 450 nm in a microplate photometer (using air as blank) within 15 minutes after the addition of stop solution to prevent fluctuation in OD values. 100 µl of positive control solution and 100 µl of negative control solution, both supplied with the kit, were added in duplicate to selected wells. OD readings from the samples were compared to those of the positive and negative control sera.

After calculating the mean OD -values for each of the controls and samples, the percent inhibition (PI) values for positive control as well as samples were calculated using the following formula:

$$PI = (OD_{Neg\ ctrl} - OD_{Sample/Pos\ ctrl}) \times 100$$

$$OD_{Neg\ ctrl}$$

Then the result was interpreted as positive, doubtful and negative, if PI was greater than 40, between 30 and 40 and less than 30 respectively. Samples with a PI value between30 and 40 were retested. A negative result was indicated by a strong color change since coated antigen remain free for HRP conjugated monoclonal antibodies directed to NDV. To ensure assay validity, the company recommended that the negative control solution should have an optical density (OD) value greater than 0.600 and the positive control solution should have a PI greater than 40.

Data Analysis: Data entry was done using Microsoft office Excel and processed using SPSS version 15 statistical soft ware after importing the data from microsoft excel. Descriptive statistics were computed for all the parameters and different PA. Chi square and Fischer's Exact tests was used to analyze the differences in the sero-prevalence between the sexes, ages, altitudes and breeds. A P-value less than 0.05 were considered to be statistically significant.

RESULTS

Out of the 355 sera from the chickens sampled, 20 were found to be positive to *Newcastle Disease Virus* antibodies. The overall sero-prevalence in this study area were therefore 5.6% (3.2-8.0 % prevalence at 95% CI). Five (62.55%) of the eight PAs sampled had chickens that were positive for antibodies against *Newcastle Disease Virus* by the blocking ELISA. The prevalences in each PAs are presented in Tables 1 and 2.

The prevalence ranges from 0 to 28.1% in the lowland Pas with an overall seroprevalence of 7.8% (4.43 _ 11.7 % at 95% CI). Only one (20%) of the PAs sampled had no positive samples. The rest 80% of the PAs sampled do have positive serum for *Newcastle Disease Virus* antibodies. The highest prevalence (28.1%) was recorded at Harbu followed by Muti, Adadi and Wenber (Table 1).

Only one PA (33.3%) from the highland had sero-positive animal but the rest two (66.6%) did not have positive chickens for *Newcastle Disease Virus* antibodies in their serum. The overall seroprevalence in highland area was only 0.9 % (0- 2.64 % at 95 CI) (Table 2).

Table 1: Seroprevalence of Newcastle disease virus antibodies in lowland peasant associations

Peasant Associations (PA)	*Positive sera*	*Negative sera*	*Total*	Prevalence
Kersa	0	27	27	0
Wenber	3	68	71	4.2
Adadi	3	65	68	4.4
Muti	4	41	45	8.9
Harbu	9	23	32	28.1
Total	19	224	243	7.8

Table 2: Seroprevalence of Newcastle disease virus antibodies in Highland Peasant Associations

Peasant associations(PA)	*Posetive sera*	*Neggative sera*	*Total*	Prevalence
Ilala	0	55	55	0
Wako	0	19	19	0
Godeti	1	37	38	2.6
Total	1	111	112	0.9

Table 3: Description of study chickens by altitude, age, sex and breed

	Altitude		*Age*		*Sex*		*Breed*	
	Highland	Lowland	Young (3-6 months)	Adult (> 6 months)	Male	Female	Local	Cross
Pos. sera	1	19	0	20	1	19	8	12
Neg. sera	111	224	81	254	50	285	288	47
Total	112	243	81	274	51	304	296	59
Prevalence	0.9	7.8	0	7.2	1.9	6.2	2.7	20.3
*x*2-value	*6.92*		*6.26*		*1.51*		*28.78*	
Significance	*p = 0.009*		*p=0.01*		*p=0.331*		*p=0.00*	

A significant ($p<0.05$) difference in sero-prevalence was found between highland and lowland areas, adult and young chickens. Furthermore, there was a highly significant difference ($p = 0.00$) in the NDV antibodies level between local and cross breeds of chickens. The difference, however, is not statistically significant ($p>0.05$) for sexes. Highest prevalences were recorded in cross breeds of chickens (20.3%) and sero-positive samples were not found in the young chickens. Only one sero-positive sample was found in the highland of the study site (Table 3).

DISCUSSION

Antibodies to NDV were identified in five (62.55%) of the eight PAs sampled, which agrees with similar studies in Botswana, Mexico and Ethiopia [5-7]. There is no history of vaccination in the study area, the chickens might have been exposed to natural infection. The presence of Newcastle disease virus antibodies in the sera of the chicken in this study was an indication of previous exposure of the chickens to the virus. Since all of the chickens sampled were over three months of age the presence of maternal antibodies can be ruled out for such antibodies are known to wan after the age of 3-4 weeks [8].

Zeleke *et al.* [5] reported an over all prevalence of NDV antibodies as 19.78% in southern and rift valley areas of Ethiopia. The overall seroprevalence of NDV antibodies 5.6% (95% CI 3.2-8.0%) found in this study were lower than the above study, but the study methods were different in that they used Haemagglutination Inhibition method for the detection of serum antibodies where as blocking-ELISA was used for this study for the detection of NDV antibodies. A serological survey conducted for NDV antibodies in village chickens in Mexico having similar study design with this study revealed that there were seroprevalence of 2.2% (95 CI 0.5-3.8%) [6], which agrees with this study.

The low prevalence or absence of detectable antibodies to NDV in the four PAs sampled indicated that the village chickens in such PAs would be highly susceptible to the pathogenic NDV infection [9]. In their serological study in Ethiopia, Zeleke *et al.*[5] found that

the chickens from two out of seven districts sampled did not have antibodies to NDV; which indicated that, even in enzootic areas, some village can remain fully susceptible to infection with virulent NDV.

The prevalence varied between sexes; however, the difference was not statistically significant which is in accordance with the previous studies [2,5]. In this study, there was a significant difference ($p<0.05$) in the sero-prevalence between altitudes. The low altitudes do have higher sero-prevalence than the high altitude which agrees with the findings of Zeleke *et al.* [5] and Tadesse *et al*. [2] who reported that there were a high prevalence in the lowland than highland even though the difference is not significant in their study. The possible explanation for this could be there are few chickens in the highland area of the study sites and chicken population number is a factor for the transmission of the disease.

The observed differences in the rates of NDV antibodies in highland and lowland may also be because of ecological variations in ND activity and may perhaps be a reflection of the impact of environment on the viability of NDV and epidemiology [10]. Other factors responsible for this difference may be as result of the presence of commercial poultry farms in the lowland. There is no poultry farm in the highland but there is one poultry farm in the lowland. Commercial poultry are routinely vaccinated against ND virus but rural household chicken may come in contact with them especially during sales. Hadipour, [11] demonstrated that the risk of back yard chicken flock being seropositive for NDV increased with the increasing proximity to the nearest neighbor poultry farm.

The difference in the seroprevalence between > 6 months and 3-6 months of age was also statistically significant ($p<0.05$), which agrees with the finding of Vui *et al*. [12] which stated that the 2- 6 months-old groups had a significantly lower NDV antibody titre than the > 6 month-old age group. 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.

A significant difference ($p=0.00$) in the seroprevalence between the lacol and cross breeds of chickns has been found in the study area which disagrees with the finding of Vui *et al*. [12]. They found that there was no significant difference between the local and hybrid chicken. The question of breed susceptibility to ND is still controversial [13]. Highest prevalence (28.1%) was recorded at Harbu PA, which is located just near to the market. The proximity of the PA to the market may be the reason for this highest prevalence.

CONCLUSION

The low prevalence or absence of detectable antibodies to NDV in the four PAs sampled indicated that the village chickens would be highly susceptible to the pathogenic NDV infection. Thus, it is recommended that there should be routine vaccination program in the study area. Highest prevalence (28.1%) was recorded at Harbu, which is located just near to the market. Within the country, NDV may spread with animal movements, such as buying, selling and exchanging poultry. The risk of markets, however, has not been evaluated and thus their role in the spread of NDV is not known. Therefore, further study should be conducted to determine the role of markets for the transmission of the diseases in the country.

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REFERANCES

1. CSA, 2008. Central Statistics Agency, Ethiopia.
2. Tadesse, S., H. Ashenafi and A. Zeleke, 2005. Seroprevalence study of Newcastle disease in local chickens in centeral Ethiopia. International Journal App Res Vet Med., 3(1): 25-29.
3. Dessie, T., 1996. Studies on village poultry production system in the central high lands of Ethiopia, M. S. thesis, Swedish University of Agricultural science, Sweden.
4. Nassir, M.H., 1998. Oral Newcastle disease vaccination trial and studies of Newcastle disease in Ethiopia, M. S. thesis, Addis Ababa University School of graduate studies and Freie Universitat Berlin, faculty of veterinary medicine, Ethiopia.
5. Zeleke, A., T. Sorri, E. Gelaye and G. Ayelet, 2005. Newcastle disease in village chickens in the southern and rift valley district in Ethiopia. International Journal of Poultry Science, 4(7): 507.

6. Gutierrez-Ruiz, E.J., I.E. Ramirez-Cruz, C. Gamboa, J.D. Alexander and R.E. Gough, 2000. A serological survey of avian infectious bronchitis virus and Newcastle disease virus antibodies in backyard (free-range) village chickens in Mexico. Tropical Animal Health and Production, 32(6): 381-390.
7. Mushi, E.Z., M.G. Binta, R.G. Chabo, J.M.K. Hyra and K.M. Thibanyane, 2001. Antibodies to Newcastle disease virus in the sera of indigenous chickens in Oodi, Kgatleng, Botswana. Onderstepoort Journal of Veterinary Research, 68: 69-70.
8. Murphy, F.A., E.P.J. Gibbs, M.C. Horzinek and M.J. Studdert, 1999. Veterinary virology. California Academic Press, pp: 411-421.
9. Allan, W.H. and R.E. Gough, 1974. A standard haemagglutination inhibition test for Newcastle disease vaccination and challenge. The veterinary Record, 95: 147-149.
10. Orajaka, L.E., D.F. Adene, B. Anene and E.A. Onuoha, 1999. Seroprevalence of Newcastle disease in local chickens from southeast derived Savannah zone of Nigeria. Rvued'Elevage et de Medicine Veterinaries des Pays Tropicaux, 52: 185-188.
11. Hadipour, M.M., 2009. A serological survey for Newcastle disease virus antibodies in back yard chickens around Maharlou Lake in Iran. Journal of Animal and Veterinary Advances, 8: 59-61.
12. Vui, T.Q., J.E. Lohr, M.N. Kyule, K.H. Zessin and M.P.O. Baumann, 2002. Antibody Levels against Newcastle Disease Virus, Infectious Bursal Disease Virus and Avian Influenza Virus in Rural Chickens in Viet Nam. International Journal of Poultry Science, 1 (5): 127-132.
13. Awan, M.A., M.J. Otte and A.D. James, 1994. The epidemiology of Newcastle disease in rural poultry: a review. Avian Pathology, 23: 405-423.
14. Hyun, J.C., C.E. Susan, G.T. Green and L.T. Jordan, 2002. A blocking ELISA with improved sensitivity for the detection of passively acquired maternal antibodies to BHV-1. The Canadian Veterinary Journal, 43(1): 43-45.