

Sero-Prevalence Of Newcastle Disease And Its Associated Risk Factors In Village Chickens At Alamata District, Southern Tigray, Ethiopia

Getachew Derbew*, Belayneh Getachew and Berihu Haftu

Belayneh Getachew (DVM, MSc, PhD), Mekelle University College of Veterinary Medicine.

Berihu Haftu (DVM, MSc), Department of Biomedical Sciences Adigrat University

Abstract - A cross-sectional study on Newcastle disease (ND) was conducted in apparently healthy and non- vaccinated village chicken at Alamata district from December 2015 to April 2016. A total of 290 chickens were randomly selected to determine the sero- prevalence and identify possible risk factors for the disease using haemagglutination inhibition (HI) test. The overall sero- prevalence of Newcastle disease in the study area was found to be 26.2% (76/214). A statistically significant difference ($\chi^2= 4.627$; $p= 0.031$) in the sero- prevalence between the young and adult chicken were found in the study area. The difference in sero-prevalence between local and cross breed was also statistically significant ($\chi^2= 21.799$; $p= 0.00$). However, the difference were statistically insignificant between sexes and flock size ($\chi^2= 4.755$; $p= 0.665$ and $\chi^2=0.603$; $p= 0.437$) respectively. The high prevalence and no vaccination history indicated that, the chicken populations in the study area have been exposed to the virus sometime in the past. Based on this result the disease affects more likely to young, cross and lowland chickens according to age, breed and altitude respectively. Therefore, further study has to be conducted to know the depth (strength) of the impact of those possible risk factors on epidemiology of this disease.

Key words - Alamata, haemagglutination inhibition test, Newcastle disease, sero- prevalence, Village chicken

I. INTRODUCTION

Poultry industry in Ethiopia is dominated by the traditional sector. Free-range poultry keeping is most common in the country. Ethiopia has an estimated of 50.38 million with indigenous chicken of non-descriptive breeds accounting 96.9%, exotic breeds 2.56% and hybrid chicken 0.54 %. Moreover, 96.9% of indigenous chickens have been distributed in different agro-ecological zones of the country [11].

In most part of Ethiopia, village chicken represents a significant component of the rural household livelihood as a source of cash income and nutrition [13]. According to the central statistical agency report of 2011 the annual egg production of the country is estimated as 98.3 million [12]. Still these large population indigenous chickens are found in traditional production systems. But, they are well adapted to the tropics, adaptable to poor management, feed shortages, tolerate to diseases and provide better test of meat and eggs than exotic chickens [16]. Furthermore, short generation interval, high rate of productivity, easy to transport in different areas and easily consumed by the rural poor community are the major opportunities of chickens comparing with other farm animals [26]. Despite of their significant roles, rearing them has been considered as side line Agricultural activity. Diseases especially the devastating Newcastle disease (ND) is perceived to be the main constraint [22] which frustrates any investment in poultry production in the country. ND is caused by virulent strains of avian paramyxovirus type 1 (APMV-1) serotype of the genus Avulavirus belonging to the subfamily Paramyxovirinae, family Paramyxoviridae. The paramyxoviruses isolated from avian species have been classified by serological testing and phylogenetic analysis into ten subtypes designated APMV-1 to APMV-10 [19]; ND virus (NDV) has been designated APMV-1 [5]. The infection takes place by inhalation or ingestion of the virus or by contact with mucous membranes, specially the conjunctiva. Infected birds shed virus in aerosol, respiratory discharge and faeces. Infected birds start to excrete virus during the incubations period and continue to excrete virus for a varying but limited time during convalescence [10]. It is enzootic in most countries in Africa, Asia and South America, where it continues to cause serious losses despite the vaccination of industrialized poultry (Aldous and Alexander, 2001). The disease causes high economic losses due to high mortality, morbidity, stress, decreased egg production and hatchability [4].

The mortality rate could be up to 100% and it is considered to be among the most important viral diseases of poultry in the world. Newcastle disease has a worldwide distribution [2]. The availability of standard sensitive serological test which is adapted to the conditions in these countries would facilitate diagnosis and accurate monitoring of vaccination programs. The haemagglutination inhibition (HI) test is used most widely in ND serology test. Its usefulness in the diagnosis depends on the vaccinal immune status of the chicken to be tested and on prevailing disease conditions [23]. The potential of the free-range chicken production has not been exploited because of diseases like ND. 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 [29]. For this to be feasible a base line data should be established to assist in formulating ND control programs. 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 including Alamata district. 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.

II. MATERIAL AND METHODES

2.1. Study area

This study was conducted from December 2015 to April 2016 in Tigray regional state at Alamata district. It is situated 180kms south of Mekelle, capital city of Tigray Regional State, and 600kms north of Addis Ababa, capital city of Ethiopia. The district has an altitude ranging from 1178 to 3148 above sea level and the mean minimum and maximum annual rain fall is 615mm and 927mm, respectively. The rain fall of the area is bimodal with the small rain (short rain season) covering 80% of the district and occurs between January to April, and main rain (long rain season) covering the whole district and occurs from June to August [17]. Agro climatically, the study area is divided into two, intermediate high land ('Dega') which covers 25% and the low land ('Kola') which covers 75% of the district Mixed crop and livestock farming system is the mode of agriculture in the study site.

2.2. Study Design

A cross sectional epidemiological study was conducted on both sexes and different age of village chicken. The study was undertaken from December 2015 to April 2016 to determine the sero-prevalence of Newcastle Disease Virus Antibodies and associated risk factors in village chickens. Purposive and systematic random sampling techniques were used to select peasant associations and households, respectively. Initially, based on accessibility to transport and difference in geographical location five peasant associations were selected. Those were Adi-shehashm, Rarhie and Adis-kigni from the low land area, and Dafat and Hanak-sraw from the high land area. Most of the chickens were local breeds and the rest were cross. The study animals were classified in to male and female, high land and low land, and young (3 up to 6 months old) and adult (greater than or equal to 6 months).

2.3. Study Subjects

The target animals were non vaccinated backyard chicken found in different villages that varies with age, sex and breed, but the same management and production system. The total chicken population in the study area was 114449 [9].

2.4. Sample Size Determination

A cross-sectional epidemiological study was conducted to determine the overall sero-prevalence of ND in the study area. The sample size was determined with relevant formula for simple random sampling at 95% confidence interval and 5% desired level of accuracy. A study conducted in southern and rift valley parts of Ethiopia on prevalence of Newcastle disease showed an overall prevalence 19.78% [29]. By taking this prevalence, the sample size was calculated based on the formula given by [28].

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

.....Where n = number of sample size

d^2 = absolute precision (5%),

P_{ex} = Expected prevalence (19.78%).

Based on the formula given, the minimum sample size was calculated to be 244. To increase the precision of the test 46 samples were added and hence the total sample size was increased to 290.

2.5. Sampling Methods

In Alamata district, there are around 114,449 Poultry population [9]. Systematic sampling method was adopted to select the 290 village chickens for sampling. Therefore, five peasant associations (PAs) were selected based on altitude i.e. three from lowland (Rarhie, Adi-shehashm and Adis-kigni), and two PAs from the highland (Dafat and Hanak-sraw). As a result the total sample size was classified in to those PAs based on proportionality sampling. Sample size of each altitude was stated in the following table 1:

Table 1. Sampling area and sex distribution of study chickens

Altitude	Male	Female	Total
Lowland	30	162	192
Highland	34	64	98

2.6. Serum sample collection

After disinfecting the blood collection site (brachial vein of chickens) with cotton soaked in 70% ethanol, a total of 3 ml of blood was collected aseptically from apparently healthy village chicken in a 5ml syringe. The syringe was placed in a slant position for some time at room temperature up to serum was formed from the collected blood. Then after that the serum was decanted into eppendorf tube for transportation by using ice box after proper labeling. The serum was centrifuged at 2500 rpm (revolution per minute) for five minute to remove and exclude the remnants from the serum and it was transferred in to another eppendorf tube. After that the serum was stored at -20°C until the Haemagglutination-inhibition (HI) test was carried out.

2.7. Methods of Data Collection

Data was collected during blood sample collection through labeling of all the basic information in each container using easily understandable code. In line with this there was data collection format which was used to get more clear information about the risk factors such as altitude, sex, age, breed and disposal of dead bird which was fulfilled at the time of blood sample collection.

2.8. Antigen production in embryonated chicken egg

Antigen production was done from the HB1 (Hitchner B1) live attenuated vaccine of NDV obtained from NVI (National Veterinary Institute) by standard method using embryonated chicken eggs. The embryonated chicken eggs of 9 to 11 days old were used for inoculation [5]. The eggs were disinfected and candled to check the viability and to delimit the air sac at the inoculation site of less number of blood vessels. The eggs were drilled manually to make a small hole about 2 mm above the delimitation of the air

sac and inoculated in allantoic cavity with 0.2 ml of diluted HB1 live attenuated NDV vaccine. Finally, the eggs were sealed with glue and incubated at 37°C for 4 days in an incubator.

The inoculated eggs were candled every 24 hours to check embryo vitality. Eggs containing dead embryo on each day and those remaining at the end of the incubation period, were removed from incubator and chilled at +4°C overnight. The next day, allantoic fluids were harvested and tested by HA test for its ability to haemagglutinate chicken RBCs [5].

2.9. Blood collection and washed red blood cell preparation

A 3 ml of Blood was collected from the wing vein of laboratory chicken using 4% sodium citrate solution at 1ml per 16 ml blood ratio as an anticoagulant. The syringe was slowly and gently shaken for complete mixing of the blood with the anticoagulant. After that the blood was transferred into a plain tube and centrifuged at 2500 rpm for five minutes. Then the supernatant was decanted without disturbing the pellet of red blood cells. Then 1x PBS (phosphate buffered saline) solution was added to fill its previous volume, and was mixed gently using micro pipette. Finally the cells were formed a pellet after being washed three times in the same manner. From this a 10% and 1% washed chicken RBCs were prepared.

2.10. Haemagglutination (HA) test and 4 HAU preparation

Allantoic fluids that were harvested from the HB1 inoculated embryos were tested for haemagglutination activity. For this purpose 25 microliter of 1x PBS solution was added in to 96 wells of microtiter plate. Then 25 microliter of fresh allantoic fluid was added in 1st well of microtiter plate and 2 fold serial dilutions were made in the plate up to its 11th well, and the 12th well was used as a negative control. 25 microliter of 1x PBS solution was added again up to 12th well of the plate. After that 25 microliter of 1% washed red blood cells (RBCs) were added up to 12th well and plates were incubated for 45 minutes at room temperature. Reciprocal of the last antigen dilution giving a clear lawn of evenly spread layer of RBCs were designated as antigen end titer. 4 Haemagglutinating unit (4 HAU) was calculated by dividing the end titration point testing antigen by 4.

2.11. Haemagglutination inhibition test

Antibody titer for NDV was determined from each serum sample using the OIE, HI test protocol as described by Alexander (2003). Briefly, 4 samples were tested in a single plate A-H for one sample in double. 25 microliter of 1x PBS solution was dispensed into all wells of a plastic 96-well microtiter plate (v-bottomed wells) and 25 microliter of serum sample was placed into the first A-H wells of the plate (sample one up to sample four) respectively. After that a 25 microliter of the 4 HAU of the allantoic fluid harvest was added to the last 12th A-D wells of the microtiter plate which serves as a negative control. The last E-H wells of the 12th well were dispensed again with 25 microliter of PBS solution and 1% washed RBC which serves as a positive control. With the aid of a multi-channel micro pipette, two fold serial dilutions of the serum were made across the plate (A1-A11). The last 25 microliter was discarded and 25 microliter of antigen containing 4 HAU was added to all the wells except to the 12th wells which serve as a negative control. NDV (HB1 attenuated live vaccine frequent passage) was used as source of antigen. Then the plate was covered with Parafilm and waited for 30 minutes for antigen antibody reaction if there was. After that 25 microliter of prepared 1% washed chicken RBC was added in to each wells using multichannel micropipette. Mixing was done gently by tapping and the plates placed on the bench at room temperature for 45 minute after which they were observed for HI test result. Finally, serum samples having an antibody titer of 1:16 (\log_2^4) and above were generally accepted as indicative of specific immunity i.e. positive [23].

2.12. Description of Variables

In this study, there were various risk factors for the occurrence of Newcastle disease which will be regarded as independent variables and an outcome variable (dependent variable) which was Newcastle disease. The possible risk factors to be studied in this disease were: Dependent variable; antibody of ND virus, and independent variables; age, sex, altitude, died bird disposal, breed and flock size.

2.13. Data management and analysis

The data collected from the study area was coded and recorded in Microsoft Excel spread sheet and then analyzed using SPSS version 20 statistical software. Descriptive statistics were computed for all the parameters and different PAs. Chi square was used to analyze the differences in the sero-prevalence between the sexes, ages, altitudes, flock size and breeds. A P-value less than 0.05 were considered to be statistically significant.

III. RESULTS

In this study a total of 290 sera were tested by HI and an overall sero-prevalence of 26.2% was recorded in the study area (Table 2).

Table 2. Overall sero-prevalence of NDV antibodies at Alamata district

Total number of sera tested	Sero positive	Prevalence rate
290	76	26.2%

From the total serum sample 226 and 64 were female and male chicken, respectively. The result revealed that the disease has no statistically significance variation between sexes ($\chi^2 = 4.755$; $p = 0.415$).

Table 3. Sero-prevalence of NDV antibodies in male and female chicken and their association

Sex	Total	Positive	χ^2 -value	p- value
Female	226	66(29.2%)		
Male	64	10(15.6%)		

Total	290	76	4.755	0.415
-------	-----	----	-------	-------

The overall sero- prevalence of NDV antibodies based on age was different in adult and young chicken. The prevalence of this disease varied significantly ($\chi^2=4.627$; $p= 0.031$) between age categories, the adult chicken (29.7%) were more affected than young chicken (17.3%) (Table 4).

Table 4.Sero- prevalence values of NDV in young and adult chicken and their association.

Age	Total	Positive	X ² - value	p- value
Young	81	14(17.3%)		
Adult	209	62(29.7%)		
Total	290	76	4.627	0.031

Total sero- prevalence of the disease in local and cross breed chicken was 17.9% and 43.6% respectively. The result revealed that the disease has statistically significance variation between breeds ($\chi^2= 21.799$; $p= 0.00$) (table 5).

Table 5.Sero- prevalence value of NDV in local and cross breed and their association

Breed	Total	Positive	X ² - value	P value
Local	196	35(17.9%)		
Cross	94	41(43.6%)		
Total	290	76	21.799	0.00

Total sero- prevalence of the disease in lowland and highland altitude chicken was 31.8% and 15.3% respectively. The result revealed that the disease has statistically significance variation between altitude ($\chi^2= 9.095$; $p= 0.03$) (table 6)

Table 6.Sero- prevalence values of NDV in lowland and highland altitude and their association

Altitude	Total	Positive	X ² - value	p- value
Lowland	192	61(31.8%)		
Highland	98	15(15.3%)		
Total	290	76	9.095	0.03

From the total sera sample 137 and 153 were have flock size of less than five chicken and six up to eleven chicken respectively. The result revealed that the disease has no statistically significance variation between flock sizes ($\chi^2= 0.603$; $p= 0.437$) (table 7).

Table 7.Sero- prevalence value of NDV in comparative low and medium flock size and their association

Flock size	Total	Positive	X ² - value	p- value
Low(1-5 chicken)	137	33(24.1%)		
Medium(6-11 chicken)	153	43(28.1%)		
Total	290	76	0.603	0.437

IV. DISCUSSION

The present cross-sectional study concerns with sero- prevalence of Newcastle disease and its associated risk factors at Alamata district. A total of 290 unvaccinated chicken sera samples were collected in the study area. Of this, 26.2% (76) was found to be positive for specific HI antibody titers of NDV (Table 2). This disclosed that ND is one of the major diseases of chicken occurring in the study area. The presence of ND antibodies in the sera of non -vaccinated chicken were an indication of previous exposure of chicken to natural infection in the field.

The observed result (26.2%) was almost similar with the findings (19.78%, 28.57% and 29.69%), from Ethiopia the sero-prevalence of ND, reported from southern and rift valley, Debre Berhan, Sebeta by [29] and [25] respectively. Another author [1] from Nigeria was found 23.6% sero-prevalence of ND in non- vaccinated local chicken which was agreed with the present study. However, this result is lower than the findings of [25] and [6] which is 38.33% and 43.68%; a sero- prevalence study at Nazareth and central part of Ethiopia, respectively. This variation in sero -prevalence of the disease might be due to difference in geographical location and seasonal variability. But the observed results were higher than the findings of [8] from Ethiopia and [15] from Mexico which was 5.6% and 2.2% of the overall sero -prevalence of ND, respectively. This might be due to the difference in study design because they use blocking-ELISA for the detection of NDV antibodies.

A statistically significant difference ($p= 0.031$) in the sero-prevalence between the young and adult chicken was found in the study area which was in agreement with the finding of Muhammad and his friends from Faisalabad, Pakistan [20]. The disease has higher prevalence in adult chicken than the young one. This variation can be suspected due to the frequent exposure of adult chickens in their earlier age to the field 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 wane after the age of 3-4 weeks [21].

The difference in the sero-prevalence between local and cross breed was also statistically significant ($p= 0.00$). This finding is agreed with the finding of Fethu, [14]. In this study the sero- prevalence of ND is higher in cross breed than local breed. But, the question of breed susceptibility to ND is still Controversial [7]. Statistically significant variation ($p= 0.03$) was observed among the lowland and highland altitudes on sero -prevalence of ND in the study area. The low altitudes do have higher sero-prevalence than the high altitude which agrees with the findings of [29]. 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 [24]. The prevalence was varied between sexes but it was not statistically significant variation ($p= 0.415$). This finding of the study was agreed with the previous studies in showing that there was no significant difference between the two sex groups, which was supported by the report, which indicated as the disease affects more likely in both sexes almost similarly [8]. The sero-prevalence of the disease was varied between flock sizes; however, the difference was not statistically significant ($p= 0.437$). This finding had an agreement with the finding of [18].

V. CONCLUSION AND RECOMMENDATIONS

The current cross-sectional study revealed that the overall sero-prevalence of ND at Alamata district was found to be 26.2%. This result and no vaccination history indicated that the chicken population in the study area has been exposed to the virus sometime in the past. In addition to this the result revealed that 73.8% of the chicken populations in the study area are at risk of infection.

The current study also showed that the prevalence of ND based on age, breed and altitude have a statistically significant variation. Based on this result the disease affects more likely to young, cross and lowland chickens according to age, breed and altitude respectively. Therefore, according to the concluding remark the following points are recommended:

- ✓ regular vaccination should be carried out in the study area
- ✓ further study has to be conducted to know the depth (strength) of the epidemiological impact of those possible risk factors on the distribution of this disease

VI. REFERENCE

- [1]. Abraham, O., Sulaiman, L., Meseko, C., Ismail, S., Ahmed, S., Suleiman, I. and Jagboro, S. (2014). Sero-prevalence of Newcastle disease virus in local chicken in Udu Local Government Area of Delta State, Nigeria. *Journal of advance agricultural research volume*, **2**:121-125.
- [2]. Alders, R. and Spradbrow, P. (2001). Controlling Newcastle Disease in Village Chicken. A Field Manual. *Australian Centre for International Agricultural Research. Monograph*, **82**, pp. 37.
- [3]. Aldous, E. and Alexander, D. (2001). Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). *Journal of Avian Pathology*, **30**: 117-128.
- [4]. Alexander, D. (2000). Newcastle disease and other avian paramyxoviridae infection; Disease of Poultry. Ames, Iowa State University Press, Pp. 541-569.
- [5]. Alexander, D. and Senne, D. (2008). Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In: Saif, Y., Fadly, A., Glisson, J., Mc, D., Larry, R., Nolan, L. and Swayne, D. (eds). *Diseases of Poultry* (12th ed.). Blackwell Publishing, Ames, Iowa State University Press, Pp 750-798.
- [6]. Ashenafi, H. (2000): Survey on Identification of Major Diseases of Local Chickens in Three Selected Agro-Climatic Zones in Central Ethiopia. Veterinary Doctoral Thesis, Faculty of Veterinary Medicine, Addis Ababa University, DebreZeit, Ethiopia. Pp. 41-45.
- [7]. Awan, A., Otte, J. and James, D. (1994). The epidemiology of Newcastle disease in rural poultry: a review. *Avian Pathology*, **23**: 405-423.
- [8]. Belayneh, G., Moses, N., Kyule, N., Melese, B. and Fufa, D. (2014). Seroprevalence of Newcastle Disease Virus Antibodies in Village Chickens in Kersana-kondalaity District, Ethiopia. *Journal of Global Veterinarian*, **12** (3): 426-430.
- [9]. CASCC (Central Agricultural Census Commission) (2013). Ethiopian agricultural sample enumeration, Results for Tigray Region. Statistical report on livestock and farm implementations, Addis Ababa, Ethiopia. Pp 29-43.
- [10]. Caupa, I. and Alexander, D. (2009). Avian Influenza and Newcastle Disease. A Field and Laboratory Manual. Milan: Springer-Verlag. ISBN 978-88-470-0825-0.
- [11]. CSA, (Central Statistics Authority). (2012). Agricultural Sample Survey; Statistical Bulletin **2**: 505 Report on Livestock and Livestock Characteristics, Addis Ababa.
- [12]. CSA, (Central Statistics Authority). (2011). Statistical Report on Farm Management Practice, livestock and Implements, Part II, CSA, Addis Ababa, Ethiopia.
- [13]. Dhuguma, R. (2009). Understanding the Role of Indigenous Chickens during the Long Walk to Food Security in Ethiopia. *Livestock Research for Rural Development*, **21**(8).
- [14]. Fethu, L. (2014). Virus isolation and seroprevalence determination among cases of Newcastle and infectious bursal diseases of chickens raised in Harar, Dire Dawa and Jigjiga town, Ethiopia. A Thesis Submitted to the Department of Biology School of Graduate Studies, Haramaya University. In Partial Fulfillment of the Requirements for the Degree of Master of Science in biotechnology. Pp. 35-36.
- [15]. Gutierrez-Ruiz, J., Ramirez-Cruz, E., Gamboa, C., Alexander, D. and Gough, E. (2000). A serological survey of avian infectious bronchitis virus and Newcastle disease virus antibodies in backyard (free-range) village chickens in Mexico. *Journal of Tropical Animal Health and Production*, **32**(6): 381-390.
- [16]. Hunduma, D., Regassa, C., Fufa, D., Endale, B. and Samson, L. (2010). Major Constraints and Health Management of Village Poultry Production in Rift Valley of Oromia, Ethiopia. *American-Eurasian, Journal of Agriculture and Environmental Science*, **9**: 529-533.
- [17]. IPMS, (Improving Productivity and Market Success) (2005). Alamata Pilot Learning site Diagnosis and Program Design. (Unpublished), ILRI (International Livestock Research Institute), Addis Ababa, Ethiopia. P.187-198.
- [18]. Mekonnen, G., Mahendra, p., Tadele, D., Wondmeneh, E., Wonda, M. and Simenew, K. (2013). Sero-prevalence of Newcastle disease in poultry under backyard system in village of Bisheftu town, Ethiopia. College of veterinary medicine and agriculture Adis Abeba University, Debrezeit, Ethiopia. *Research Journal of Poultry Science Volume*, **6**:38-42.
- [19]. Miller, P., Afonso, C., Spackman, E., Scott, M., Pedersen, J., Senne, D., Brown, J., Fuller, C., Uhartm. M., Karesh, W., Brown, I., Alexander, D. and Swayne, D. (2010). Evidence for a New Avian Paramyxovirus Serotype-10 Detected in Rockhopper Penguins from the Falkland Islands. *Journal of Virology*, **84** (21): 11496-11504.

- [20]. Muhammad, Y., Muhammad, A., Muhammad, S. and Iftikhar, H. (2013).Epidemiology of Newcastle Disease in Rural Poultry in Faisalabad, Pakistan.Department of Microbiology, University of Agriculture Faisalabad, Pakistan, *international journal of agriculture and biology volume,4*: 491-497.
- [21]. Murphy, F., Gibbs, E., Horzinek, M. and Studdert, M. (1999).Veterinary virology. California Academic Press, Pp: 411-421.
- [22]. Nwanta, J., Egege, S., Alli-Balogun, J. and Ezema, W. (2008). Evaluation of prevalence and seasonality of Newcastle disease in chicken in Kaduna, Nigeria.*World's Poultry Science Journal*, **64**: 416-425.
- [23]. OIE (Office of International Epizootes) (2012).Newcastle Disease.Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Paris, France, chapter 2.3.14.
- [24]. Orajaka, E., Adene, F.,Anene, B. and Onuoha, A. (1999).Seroprevalence of Newcastle disease in local chickens from southeast derived Savannah zone of Nigeria. *Rvued'Elevageet de Medicine Veterinaires des Pays Tropicaux*,**52**: 185-188.
- [25]. Serkalem, T., Hagos, A., Zeleke, A. (2005).Seroprevalence Study of Newcastle Disease in Local Chickens in Central Ethiopia.*International Journal of Applied Research in Veterinary Medicine*, **3**(1): 45-53.
- [26]. Sonaiya, E. and Swan, S. (2005). Small-scale poultry production, technical guide manual.FAO Animal Production and Health 1.Food and Agriculture Organization of the United Nations (FAO), Rome.
- [27]. Tewari, S., Aloba, E. and Nawathe, D. (1992).Detection of haemagglutination inhibition antibodies against Newcastle disease virus in unvaccinated indigenous chickens in Maiduguri, Borno state, Nigeria.*Journal ofReview Science and technology office of international diseepizootics*, **11**(3): 813-817.
- [28]. Thrusfield, M. (2007).Veterinary Epidemiology, (3rded.). Black Well Science Ltd., Pp 182-198.
- [29]. Zeleke, A., Sorri, T., Gelaye, E. and Ayelet, G. (2005).Newcastle disease in village chickens in the southern and rift valley district, Ethiopia..*Journal of International Poultry Science*, **4**(7): 507.

