academicJournals

Vol. 6(1), pp. 7-12 January, 2014
DOI: 10.5897/JVMAH2013.0229
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http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Evaluation of the newcastle disease antibody level after vaccination regimes in chickens in Debrezeit Agricultural Research Center, Ethiopia

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Accepted 8 October, 2013

Evaluation of the Newcastle disease (NCD) antibody level after different vaccination regime was conducted on 110 chickens: (32%) vaccinated and kept separated, (25.2%) unvaccinated and kept with vaccinated, (25.2%) were control groups. Four vaccination regime of chicken against NCD using live lentogenic stain, Hithcner B1 (at the age of 3 day old) and lasota (at the age of 27, 63, and 112th days of age) were used. The overall antibody level of ND in examined chickens using HI test was $Log_2^{4.42}$ in unvaccinated and mixed with vaccinated birds, $Log_2^{5.2}$ in vaccinated and mixed with unvaccinated chickens, $Log_2^{2.6}$ in control groups and $Log_2^{5.3}$ in vaccinated and kept separately. On the other hand chickens vaccinated four times at 3, 27, 63 and 112 days were found to be protective as that of common vaccination schedule (0, 18, 72, 132 and 216 day old age) in antibody level of Newcastle disease among different vaccination regime and frequencies. The result of the present study indicated that the protective antibody titter response was produced from the vaccination; hence, it is very crucial to vaccinate chickens with the full dose of vaccines against NCD in order to keep protected poultry population.

Key words: Newcastle disease, Newcastle disease virus, antibody titer, chickens, vaccination, response.

INTRODUCTION

Ethiopia is estimated to have about 57 million chickens, where the majority of them are being reared under the traditional (extensive) system. This system is characterized by a very little input from veterinarians and poultry farmers that also accounts for its small out put in terms of poultry egg and meat yield. The lack of attention given to the local chickens has forced them to roam and forage around their living premises to feed for themselves as well as to perch on higher places near human dwellings in search of shelter. It was reported that the hatchability of the eggs from local chickens is relatively high though the mortality after hatching is also immensely high which could have been avoided by proper disease prevention and husbandry measures (Serkalem, 2001).

Poverty and protein deficiency is manifested by wide spread malnutrition in children and women in village communities (Nassir, 1998; Tadesse et al., 2005). Though neglected in the development themes for a long

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time, recently many researchers and development agents believe that village chicken production play a major role in poverty alleviation and food security at household level. It provides off-farm employment and income generating opportunity and serves as a source of gifts and religious sacrifices (Rehmani and Spradbrow, 1995). The chicken also serve in waste disposal system by converting leftover of grains (Rehmani and Spradbrow, 1995) and human foods and insects into valuable protein foods (egg and meat) (Kirkland, 2000).

The first documented evidence of ND in Ethiopia dates back to 1971 in an area that is now known as the country of Eritrea**.** The NDV (Newcastle disease virus) involved was a velogenic strain and caused some 80% mortality. How the virus was introduced into the country is still unknown. Then the disease spread to other parts of the country at tremendous speed. Vaccination against the disease was not practiced until 1974. Since 1991 the national veterinary institute (NVI) has produced more than 12 million dose of vaccine, half of which was sold to commercial poultry farms (Yohannes, 2008).

Poultry diseases are considered as the most important constraints responsible for reducing both the number and productivity. The current disease related mortality from egg to adult chicken is estimated to be 20 and 50%. During some spectacular epidemics mortality high as 80% were recorded and further, loss of production of surviving birds must be noted (Almargot, 1987).

Newcastle disease in commercial or village chickens is a problem through out the year although it is more serious at the beginning of the rainy season (Nassir, 1998). In many developing countries Newcastle disease (ND) appears to be the most important avian disease. Outbreaks of ND unpredictable and discourage villager from paying proper attention to the husbandry and welfare of their chickens (Spradbrow, 2000). Newcastle disease (ND) is worldwide in distribution (OIE, 2008) and regarded as one of the most economically important diseases of poultry and other birds; because of devastating consequences of Newcastle disease virus (NDV) (Wambura ,2002) called avian paramixo virus serotype 1-(APMV-1). The virus has single stranded RNA that can be categorized in to three groups: the velogenic strain, mesogenic strain and lentogenic strain. ND is enzootic in some areas of the world, especially where rural chicken breeding is dominant (Jordan et al., 2001). Human infections have also been reported among laboratory workers (Echeonwu et al., 2007).

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 (Spradbrow, 1993).

ND control include strict quarantine, slaughter and disposal of all infected and exposed birds, and disinfection of the premises, but ND control through vaccination is generally a very cost effective intervention and given a high priority by farmers (Alexander et al., 2004). Vaccination has been considered the most effective means of controlling ND and has been used successfully through out the world since the 1940s (Dias et al., 2001). Vaccinations should be thought of as insurance. Like insurance, there is a price to be paid for the protection against a potential threat. Costs include price of the vaccine, time spent designing the vaccination schedule and paying for the crew that administers the vaccines. Another major cost for vaccination, which is rarely considered, is due to the losses from vaccine reactions from the live type vaccines and local tissue reactions associated with the inactivated vaccine injections (Dias et al., 2001).

Vaccine quality is commonly blamed when a disease occurs; however, there are usually other factors responsible such as lack of a cold chain. A comprehensive investigation is often called for to identify the cause(s) and to resolve the problem (Wambura, 2000). In Ethiopia, two types of vaccines which have been in use are: (1) Conventionally used vaccines which comprise: Hitchener B1(HB1) and LaSota live freeze dried vaccines produced in 500 and 100 dose vials, produced by NVI, Debre zeit, Ethiopia and (2) thermo stable vaccine. The thermo stable vaccine NDV I 2 is also live freeze dried, produced in 500 dose vials (Tadele, 1996). This is a non-pathogenic heat resistant vaccine, transportable without freeze and given orally with feed grain without catching birds (Wambura, 2000).

Vaccines are used to prevent or reduce problems that can occur when a poultry flock is exposed to field disease organisms. Vaccinations should be thought of as insurance. Like insurance, there is a price to be paid for the protection against a potential threat. Costs include price of the vaccine, time spent designing the vaccination schedule and paying for the crew that administers the vaccines. Another major cost for vaccination, which is rarely considered, is due to the losses from vaccine reactions from the live type vaccines and local tissue reactions associated with the inactivated vaccine injections. Vaccine quality is commonly blamed when a disease occurs; however, there are usually other factors responsible. A comprehensive investigation is often called for to identify the cause(s) and to resolve the problem (Alexander and Westbury, 2001).

There are two methods used to measure antibody

titers: the heamagltination inhibition (HI) test and the enzyme linked immunosorbent assay (ELISA). The most commonly used method is HI test. The HI titer is the reciprocal of the highest dilution of serum which completely inhibits haemaglutination and is usually and most conventionally expressed as the logarithm to the base 2. Although the test is difficult to standardize between laboratories, the HI titer gives an indication of the immune status of the bird. Sequential samples taken at different times can indicate whether the titer is rising or declining (Echeonwu et al., 2007; Alexander et al., 2004). Many trials have been conducted to develop a single annual vaccination program that can significantly control ND and reduce the vaccination cost. In Ethiopia various vaccines are available commercially for the control of ND. Therefore the objective of the study was to determine the protection level of the vaccine among vaccinated chicken; design and introduce new vaccination schedule and measure the protective level in unvaccinated mixed and separated group.

MATERIALS AND METHODS

Study area and animal

Study was conducted at Debrezite agricultural research institute which is located about 45 km south east of Addis Ababa at the altitude of about 1850 meters above sea level. One hundred and ten chicks were randomly split into four treatment groups; tagged (26 in number and receive vaccine based on the schedule), untagged (26 in number and doesn't take vaccine but kept with vaccinated chicken), vaccinated (took vaccine based on schedule and kept alone) and unvaccinated (control chicken doesn't take vaccine and kept away from those vaccinated). The chickens were vaccinated four times at 1, 3, 8 and 16 weeks of age through ocular.

Management of experimental house

The brooder house which is found in debrazite agricultural research institute was used as experimental house with 12 m^2 used for vaccinated, unvaccinated, vaccinated and mixed and unvaccinated and mixed chickens. The experimental house was thoroughly washed with water and also sprayed with 10% of formalin. After drying, clean new litter was spread over the floor, equipments including waterier, feeders was cleaned, disinfected and introduced to the house.

Management of chicken

All experimental chicken were brooded in one house until 30 days old and chickens randomly split into treatment during brooding the room and brooder temperature was maintained with a source of 500 watt bulb per bird. Water and feed was provided adequately, ration feed was obtain from their own farm. The chickens were visited by the veterinarian every day in addition to the brooder house guard.

Vaccine

Two types of vaccine were recommended for this experiment, these were HB1 and laSota strain vaccine; strain live freeze dried vaccine in 500 and 100 dose vial produced by NVI, Debrezite, Ethiopia. Within their life time the chicken was not only taking NDV vaccine, it was also taking different type of vaccine like, Infectious bursa disease virus, fowl typhoid, fowl pox at a given period of time.

Source of eggs and incubation procedures

Fertile chicken's eggs were harvested from the farm. Before incubation the egg was cleaned with 10% of formalin and checked for the size of air sac and dead embryos. Initially, eggs were incubated in an incubator at 37°C and relative humidity of 60 to 70%. The egg were incubated for 18 days, after 14 days incubated egg was candled to separate the fertile from non-fertile eggs and discard abnormal air sac size or position candling performed in dark room using Candler.

Vaccination of chickens using NDV HB1 and NDV laSota strain vaccine

The chickens used for experimental purpose were obtained from the institute farm. HB1 was used to vaccinate 3 day-old chicks and the same technique was followed for revaccination by the La Sota strain on 27, 63,112 day-old chicks. Both HB1 and LaSota vaccines were reconstituted at a rate of 100 dose/L of water and one drop of suspension (40 µl) was inoculated into one eye.

Research methodology

On the station, 110 chickens were screened by checking the protective antibody level. This was conducted by grouping them in to different experimental group.

Serology

Serum sample collection: Bleeding was done prior to vaccination, in order to obtain base line information on maternal immunity and the declining level of passive acquired immunity, from there on bleeding was carried out within interval, bleeding was carried out from all experimental chicken until the end of the experiment. This was to get information on the development of immunity after the series vaccination.

Method of serum collection: The bleeding was done by exposing and plucking feathers from the ventral surface of humeral region of the wing. Then the skin wetted with 70% alcohol and the needle which contain Alseaver solution was inserted into the wing vein, with 1 to 2 ml of blood collected and placed in vacuum tube, the sample was held at 37°C for several hours, the sample was left overnight before the serum was removed, that is if the serum was not tested immediately. This was store at -20°C until the one antibody detection mechanism called heamagglutination inhibition test was performed (OIE, 2009).

Heamagglutination inhibition test: Re and post vaccination sera was tested to see whether there was a response in antibodies after vaccination using the heamagglutination inhibition test. The test

was performed following the method described in OIE (2009) manual. Four haemagglutination (HA) unit, 1% chicken erythrocyte suspension and two fold serial diluted sera starting 1:2 were used. The antibody level for each serum sample was recorded.

Data analysis

The effects of vaccination delivered with different chicken were summarized. Then the data from the Microsoft excel sheet were processed and analyzed by using a statistical software program (SPSS version 16 ©2007 SPSS inc).

RESULT

One hundred and ten chickens were randomly split in to four groups, these were vaccinated based on schedule which are 26 (25%), unvaccinated chickens which were mixed with vaccinated chickens (26) (25.2%). The control groups which are unvaccinated and kept separately were 26 (25.2%), vaccinated and kept separately were 32 (31.1%). The overall antibody titters of Newcastle disease in vaccinated chickens (51%) (≥1:8) was 100%, which indicates that all vaccinated chickens that receive the vaccine based on new schedule were totally protected (Table 1).

The new vaccination schedule was conducted in the 3rd, 27th, 60th, and 112th day. The birds which were vaccinated and unvaccinated were shown antibody titter up to 15 days, antibody titer above (≥1:8). This means the birds have maternal derived antibody. The 16 to 30 days was unvaccinated and mixed with vaccinated birds 3.6 (±1.4), vaccinated mixed 4.9 ±1.8SD, vaccinated separated 4.4 ±1.3SD and control groups 1.6 (±0.5SD). The average antibody titer of birds examine between 31 to 15 was unvaccinated and mixed with vaccinated 5.3 ± 1.4 SD, vaccinated mixed 5.5 ± 2.3 SD, vaccinated and mixed 5 ± 0.9 SD and control groups 1.5 ± 0.7 SD. The average antibody titer of birds examine between 76 to 126 days was unvaccinated and mixed with vaccinated birds 3.3 ± 1.7SD, vaccinated and mixed 5.3 ± 0.9 SD, vaccinated and separated 5 ± 2.3 and control groups 1.5 ± 0.6 SD (Table 2).

The average antibody titter that were birds that mixed with vaccinated birds 23(88.5%) was ≥ 1:8 and 3 (11.5%) was < 1:8 and the birds that were unvaccinated and kept separately 9(34%) was ≥ 1:8 and 17 (65.4%) was < 1:8 (Table 3).

DISCUSSION

Newcastle disease is highly contagious and commonly fatal viral poultry disease affecting mainly domestic and wild avian species. The overall vaccinated chicken population which receive vaccination according to the new schedule in the study all had (100%) protective antibody titter (≥1:8) against NDV. The highest number of protected population in chicken vaccinated four times (3, 27, 63 and 112) due to the fact that booster dose vaccination of NCD was applied on chickens (Al-Garib, 2003). Based on the result of this study NCD average logarism antibody level response, vaccination of 3 days old chicken against NCD using the vaccine of Hitchner B1 (at the age of 3 days) and laSota strain vaccine at the age of (27, 63,112 days of age) produced from national veterinary institute, Debrezite, Ethiopia formed protective antibody level. Protective antibody level greater than 1:8 was detected in chickens vaccinated based on the newly designed program.

Table 1. Antibody titer of vaccinated chickens.

Variable	Mixed group	Separated group
	Vaccinated	Vaccinated
	N=26(%)	N=32(%)
Antibody titer (≥1:8)	26 (100)	32 (100)
Antibody titer (<1:8)	0 (0)	0 (0)

The most commonly used NCD vaccination program is giving vaccine to chicken at 0, 18, 72, 132,192 and 216th day old and between four month intervals, but the finding of this study showed that vaccination of chickens at 3, 27, 63 and 112th days of age was as protective as commonly used vaccination schedule (0, 18, 72, 132 and 216 days so that from economic point of view, the new vaccination schedule reduces cost of vaccine (transport and handling cost), labour cost and time. The less frequently that chicken have to be vaccinated the more efficient the strategy (Alders et al., 2001).

Antibody titter of chickens, which were unvaccinated and vaccinated, was protective until 18 days. So that vaccinating birds at 3 days of age was protective as that of vaccinating at day old. This is because the antibody that come from mother is protective until 18th day. After administration of the vaccine, immunity does not develop immediately, one to 2 weeks is required for a full immune response to occur (Dias et al., 2001). Vaccination at 27, 63 and 112 days is protective enough. This is due to the fact that booster dose vaccination of NCD was applied on chickens (Al Gabi, 2003). The average logarism antibody titer birds that were mixed with vaccinated birds 23 (88.5%) was ≥ 1:8 and 3 (11.5%) was < 1:8 and the birds that were unvaccinated and kept separately 9 (34%) was ≥ 1:8 and 17 (65.4%) was < 1:8. Therefore based on study findings, 23 (88%) of birds that were mixed with vaccinated birds had got protective average antibody titter ≥ 1:8 that was highly significant (< 0.05) and only 9

Table 2. Average antibody titer of examined chickens.

Group of study animal	Average antibody titer + SD /log_2/ 1 to 15 days of vaccination	16 to 30 days of vaccination	31 to 75 days of vaccination	76 to 126 days of vaccination	Total
Unvaccinated (mixed)	5^a±1.5(7)	3.6^a±1.4(7)	5.3^a±1.4(8)	3.3^a±1.7(4)	4.4±1.3SD
Vaccinated (tagged)	5.1^a±1.9(7)	4.9^a±1.8(7)	5.5^a±2.3(8)	5.3^a±0.9(4)	5.2±1.9SD
Unvaccinated (control)	4.67^a±1.3(9)	1.6^b±0.52(11)	1.5^b±0.7(2)	1.5^b±0.6(4)	2.62±1.7SD
Vaccinated (separate)	6.3^a±0.5(12)	4.4^a±1.3(7)	5^a±0.9(9)	5^a±2.3(4)	5.3±1.3SD

a = logarithm average antibody ≥ 1:8 ($Log2^3$) which are protected. b = logarithm average antibody < 1:8 (< $Log2^3$) which are not protected.

Table 3. Antibody level of unvaccinated chickens

Variable	Mixed group	Separated group
	Unvaccinated	**Unvaccinated (control)**
	N= 26(%)	**N= 26(%)**
Antibody titer (≥1:8)	23 (88.5)	9 (34.6)* $p < 0.05$
Antibody titer (<1:8)	3 (11.5)	17 (65.4)

(34%) birds that were kept separately had got protective antibody titter. The live NCD vaccine spreads from vaccinated to unvaccinated birds when housed together. This is because of excretion of vaccine virus by these chickens evidently sufficient to re-infect the birds and boost their titter of antibody (Spradbrow, 1994).

The current study showed that ocular vaccination of chickens based on the newly designed schedule induced protective antibody level that can protect birds from NCD outbreak. When birds were mixed with vaccinated birds, they acquire the vaccine virus and their protective antibody increases, so that they can survive in case of outbreak.

ACKNOWLEDGMENTS

Authors would like to thank DZARC to granting the permission to undertake this study and we also want to thank Dr.Tadios Habte for providing the facilities used for this research.

ABBREVIATIONS

APMV, Avian paramixo virus; **CSA,** Central Statistic Agency; **EDTA,** ethylene diaminetetra acetic acid; **HB1,** hithner B1; **HI,** heamagglutination inhibition; **HN,** heamagglutinin neuraminidase; **IgA,** immuno globulin A; **IgG,** immuno globulin G; **NDV4-HR,** Newcastle disease heat resistance V4; **NVI,** National Veterinary Institute; **OIE,** Office International Des Epizootics; **PBS,** phosphate buffer saline; **RBCs,** red blood cells; **SPF,** specific pathogen free; **SPSS,** statistical package for social science; **VN,** virus neutralization.

REFERENCES

Alders R, Spradbrow PB (2001): Controlling Newcastle in rural Nigerian Veterinary Journal 2010 Vol. 31(1):46-55 54.

Alders RG, Fringe R, Mata BV (2001): Characteristics of the I-2 Live.

Alexander DJ, Allan WH (1974): Newcastle disease virus phatotype Avian pathol. 3:269-275.

Alexander DJ (1989): Newcastle disease .In: Alabratory manual for the isolation and Identification of avian patholgens. 3rd Ed, purchase

Alexander DJ (1991). Newcastle disease. In: Rweyemamu, M.M., Palya, V., Win, T. and Sylla, D., Ed. Newcastle Disease Vaccines for Rural Africa. Debre Zeit, Ethiopia, Pan African Veterinary Vaccine Centre, 7:45.

Alexander DJ (2000). Newcastle disease and other avian paramy-xoviruses.Review of Science and Technology; 9:443-462.

Alexander DJ (2001): Newcastle disease The Gorden Memorial Lecture. Br. Poult. Sci. 42:5-22.

Alexander DJ Bell JG, Alders RG (2004). Technology review: Newcastle disease with special emphasis on its effect on village chickens. Rome: FAO

Al-Garib S (2003). Newcastle disease virus: immune reactivity and pathogenesis, PhD thesis, Utrecht University, Faculty of Veterinary Medicine, The Netherlands

Almargot J (1987): Avian pathology of industrial farms in Ethiopia. First national livestock improvement conference Addis Ababa. Agricultural research institute in IARproceedings (Ed.), pp 114-117.

Dias PT, Alders RG, Fringe R, Mata BV (2001). Laboratory and field trials with thermostable live Newcastle disease vaccines in Mozambique. In: Alders RG, Spradbrow PB, ed. *SADC Planning Workshop on Newcastle Disease Control in Village Chickens.* Proceedings of an International Workshop, Maputo, Mozambique, 6-9 March, 2000. ACIAR Proceedings No. 103, pp. 91-96.

Echeonwu GON, Iroegbu CU, Echeonwu BC, Ngene A, Olabode AO, Okeke OI, Ndako J, Paul G, Onovoh EM, Junaid SA, Nwankiti (2007): Delivery of thermostable Newcastle disease (ND) vaccine to chickens with broken millet grains as the vehicle. Afr. J. Biotechnol. 23**:** 2694-2699.

Jordan F, Pttiison M, Alexander D, Faragher T (2001): Poultry disease. 5th Ed. W.B Saunders, London, pp. 257-268.

Kirkland PD (2000): virulent ND virus in Australia:in through the back door. Austral Vet: 331-333.

Nassir MH(1998): Oral Newcastle vaccination trial and studies, ND in Ethiopia, Msc thesis, Addis Ababa University, school of graduate studies and Freie University Berlin, faculty of veterinary medicine.

OIE (2008): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 6th Ed. Office International des Epizooties.

OIE (2009). Diagnostic Techniques.In: Newcastle disease .Terresterial Manual, pp.557-86.

Rehmani SF, Spradbrow PB (1995):Receptors for the V-4 strain of NCV in the digestive tract of chickens. Vet. Microbiol. 46:43-46.

Serkalem T (2001): Avian tuberculosis: preliminary study on back yard chickens reared under extensive management system in three selected agro-climatic zones in central Ethiopia.

Spradbrow PB (1988). Geographical distribution. In: Alexander, D.J., ed. Newcastle disease. Boston, MA, Kluwer Academic Publishers, 247.

Spradborow PB (1990): Village poultry and preventive Veterinary medicine . Prev. Vet. Med. 8**:**305-307.

Spradbrow PB 1993-94. Newcastle Disease in Village Chickens. Poultry Science Review, 5, 57-96.

Tadesse S, Ashenafi H, and Zeleke A. (2005): Seroprevalence study of Newcastle disease in local chickens in central Ethiopia. Int. J. Appl. Res. Ve. Med. 3(1):25-29.Thermostable Newcastle Disease Vaccine Produced at Inive. Aciar.

Wambura PN, Kapaga AM, Hyera JMK (2000): Experimental trials with atermostable Newcasle disease virus (strain I-2) in commercial and village chickens in Tanzania. Prev. Vet. Med.

Wambura PN, Kapaga AM, Hyera JMK (2000) Experimental trials with a thermostable Newcastle disease virus (strain I2) in commercial and village chickens in Tanzania. Preventive Veterinary Medicine. 2000; 43(2):75–83. doi: 10.1016/S0167-5877(99)00089-6.

Westbury HA, Parsons G, Allan WH (1984): Comparison of the residual virulence of Newcastle disease vaccine strains V4. Hithcner B1and Lasota. Australian Vet. J. 61:247-249.

Yohannes G (2008): Prevalence and risk factors of New castle disease in back yard local chickens in eastern Showa zone of oromia Regional state (DVM thesis) Faculty of Veterinary Medicine , AAU, Debre Zeit, Ethiopia.