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On Station Evaluation of Thermo-Stable Newcastle Disease Vaccine

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I. Introduction

The fact that almost all of the poultry in Ethiopia comprises indigenous (i.e., local) birds reveals that the poultry subsector is strongly dominated by small-scale, household-level chicken production. According to Tadelle *et al*. (2003) and un-published document from National poultry research program, small-scale, village poultry production in Ethiopia contributes more than 98% of the national egg and poultry meat consumption, although this figure recently might have been changed slightly due to the emerging commercialization in peri-urban agriculture.

The indigenous flocks are said to be disease resistant and adapted to their environment. However, the survival rates of the Ethiopian indigenous chicks kept under natural brooding conditions considered low. Disease and predators are known to be the major causes of mortality in the country (Holye, 1992; Negussie, 1999). According to Negussie (1999), Newcastle disease accounted for the largest proportion of overall flock mortality to be 57.3% followed by fowl pox 31.6%, coccidiosis 9.4% and predator loss 1.7%.

In Ethiopia Newcastle disease (ND) appear to be the most challenging avian disease. The disease is capable of causing 90-100% mortality in unprotected flocks (Serkalem *et. al.,* 2005), (OIE, 2013). How the virus was introduced into the country is still unknown. The disease is transmitted from bird to bird and from farm to farm mainly via aerosol but contaminated feed and water, feces from sick bird and egg and carcass from infected birds are also means of transmission (OIE, 2012).

The common control strategies of the ND around the globe are vaccination, strict quarantine, slaughter and disposal of all infected and exposed birds and disinfection of the premises. Vaccination is generally very cost effective intervention and given a high priority by farmers in most developed nations where infrastructure and veterinary service are well known/available (Alexander *et al*., 2004).

Vaccination has a cost 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). Many trials have been conducted to develop village vaccination program and reduce cost of Newcastle disease vaccination for scavenging poultry production system (Nasser *et.al.* 2010).

Having thermostable vaccine is enabling farmers to worry less on the logistics related with the cold chain. The immunogenicity of the thermostable I2 vaccine was mentioned by works of Nasser *et.al.* (2010) in ethiopia and Tu *et.al.* (1998) in Vietnam. Both report that chickens vaccinated with I2 vaccine with different route of vaccination and feed grains as a channel has a good protection effect. Many routes of vaccination were tested and their effectiveness is evaluated but the practicality of those vaccines in the country is not well disseminated in the farming community. This might be related with poor veterinary service and less practicality of the vaccination routes by rural community of the country. Therefore, the following trial on routes of vaccination was carried out in two breeds of chickens with the following objectives

a) Objective

- Determine the protection level of the litter based I2 thermo-stable Newcastle disease vaccine as compared to I2 in parboiled barley and water

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- Compare between the litter based I2 thermo-stable Newcastle disease vaccine systems with intra ocular.

II. Materials and Methods

a) Study Area

This experiment was conducted at Debre Ziet Agricultural Research Center (DZARC), Ethiopia.

b) Management of Experimental House and chickens

The experimental house and pens were thoroughly washed with water and sprayed with 3% of formalin. Separate pens were used for all treatment groups and control. After drying, clean new litter was spread over the floor. Equipments including waterier, feeders was cleaned, disinfected and introduced to the house. During brooding the room and brooder temperature was maintained with 250W infrared bulb per treatment group. Clean water and formulated feed was provided according to their requirement.

c) Experimental Design

The experiment used 190 local (Horo ecotype) and 295 KoeKoeK (South African breed) one day old chicks. The chicks were hatched at the Research center and proper hatching procedure was used. Ten sampled chicks were sacrificed and serum sample was collected at day one from both breeds. Blood sample was also collected again from 10 chicks at day 14 and 20 from both breed in order to get information on the level of maternal antibody. The remaining 180 chicks from local (indigenous) chicken and 285 chicks from koekoek breed were divided in to 15 equal groups. A single treatment has three replications. In this setup 4 treatments and 1 control were used. The sample size per treatment was calculated based on RCT sample size calculation (Chan 2003)

Table 1 : Experimental setup for I2 ND vaccination trial and viral challenge

RX	Breed	No. chicken	vaccinal viral dose	Days of vaccination*	Challenged chickens	challenge viral dose, (IM)
Eye-drop	Horro	36	10^6	21 & 36	10	10^9HA unit
	Koekeok	57	10^6	21 & 36	10	10^9HA unit
Water	Horro	36	10^6	21 & 36	10	10^9HA unit
	Koekeok	57	10^6	21 & 36	10	10^9HA unit
Feed	Horro	36	10^6	21 & 36	10	10^9HA unit
	Koekeok	57	10^6	21 & 36	10	10^9HA unit
Spray	Horro	36	10^6	21 & 36	10	10^9HA unit
	Koekeok	57	10^6	21 & 36	10	10^9HA unit
Naive	Horro	36	10^6		10	10^9HA unit
	Koekeok	57	10^6		10	10^9HA unit

d) Vaccination

The experiment uses different route of vaccination on the four treatment groups. The treatments mentioned below were given twice in 15 days interval at day 21 and 36. The vaccine was purchased from National Veterinary Institute. The vaccination was carried out after 3 weeks of age to override effect of maternal immunity (Nasser. *et al.,* 2010).

Eye vaccination: individual chicken base one eye one drop method was used to vaccinate though eye drop. A sterile standard pastor pipette was used.

Water vaccination: A water vaccination is given to chicken using distilled water. The chicks were kept without water for 2.5 hours prior to vaccine administration. It uses 10 ml per bird in the first vaccination and 20 ml per bird in the second vaccination (NVI manual).

Feed vaccination: Parboiled barley preparation was adopted from Nasser *et al.* (2010). One kg of grain is added to 1.75 litres of boiling water and left for 5 minutes. It was cooled using water. The grain was then sun dried and cracked manually. Then 1 kg with 4 liters of water twice in a day and leave it soaked overnight.

Then dried it using sunlight and use it for the treatment. The prepared barley was then sprayed using a fine sprayer in the ratio of 1 ml per 10 gram of grain. The feed was given to the birds by calculating 10 gram of feed per bird. This shows that the dose of the virus required for a single bird was calculated per 1 ml of the reconstituted vaccine.

Litter spray vaccination: I2 vaccine was used to spray the litter where experimental chicken were kept. A 1 ml per bird ratio was used in each breed.

e) Serum Collection and Haem-agglutination Inhibition Test

Blood sample was collected at day 1, 14, 20, 36, 44, 51, 58, 65 and 82 before and after vaccination. In average 1-2 ml of blood was collected in each bleeding days. Scarification was used in DOC but jugular vein and brachial vein were used in other age groups to take blood. The collected whole blood was labeled and allowed to clot under normal atmospheric condition. Then, the clear serum was harvested into labeled cryovials and stored at -20°C until HI test was carried out. The challenge virus was administered at day 65. Post challenge bleeding was done on survivors of the deadly velogenic viral challenge. The collected sera at pre and post vaccination and post challenge were tested using heamagglutination inhibition test. The test was performed following the method described in OIE (2009) manual for hemaglutination and inhibition test and the protocol of national veterinary institute (NVI). The antibody level for each serum sample was recorded using well designed recording sheet.

f) Challenge with Virulent Field Virus

Wild virus was collected from chicken embryo at NVI vaccine quality laboratory. The wild virus which collected from Haromaya by NVI was tested for haem-agglutination before administration to the birds in order to check its potency. Ten chickens from each treatment was isolated and challenged at day 65 with wild strain of ND virus. The challenge viral dose was in accordance with the work of Darminto and Daniels (1992) and Khalafall *et. al.,* (2004). The virus was given via Intra Muscular route in the breast muscle (Khalafall *et. al.,* 2004) and (Nasser *et al.*, 2010). The birds were kept under close observation for 15 days. Numbers of dead and live birds was recorded. Standard bio-security measures like restriction of movement, proper disinfection and disposal of dead chicken were implemented in-order to prevent the spread of disease.

g) Pathogenicity Index Measurement

The pathogenicity index for the challenge virus was measured using tools adopted from Tizard, 2004. The pathogenic index was set based on the time taken until an event is occurred in individual animal. To follow individual chicken each chicken was wing tagged. Then the chickens were followed for 15 days and occurrence of an event is recorded. For pathogenic index measurement four (4) categories were used according to Tizard (2004) and Mishra *et al.*, (2001). Category 0 (Zero) was given to the chicken when there was no any clinical signs; 1 (one) for in appetence and depression, 2 (two) for discharges and nervous signs, and 3 (three) for death.

III. Results

a) Hi titter in experimental animals

The result shows that there was no a significant difference in the antibody response between breeds (Table 2). But there was a significant difference between treatments (table 3).

Table 2 : Protective HI titter between two breeds

	Sum of Squares	Df	Mean Square	F	P
Between Groups	7.202	1	7.20	2.25	.134
Within Groups	1333.795	418	3.19		
Total	1340.998	419			

Table 3 : HI titters between treatments after vaccination

Treatment (1)	Treatment (2)	Mean Difference (1-2)	Std. Error	Sig.
Ocular	Water	18.889*	5.157	.003
	Feed	18.444*	5.157	.004
Water	Feed	-.444	5.157	1.000
Spray	Ocular	-3.056	5.157	1.000
	Water	15.389*	5.157	.030
	Feed	15.833*	5.157	.023
	Naïve	43.341*	4.969	.000
Naïve	Ocular	-46.397*	4.969	.000
	Water	-27.508*	4.969	.000
	Feed	-27.952*	4.969	.000

b) Pathogenic index

There is no significant difference (P= 0.82) in the pathogenic index between breeds but there is significant difference between treatments. The result shows control groups were the first in exhibiting the disease outcome in shorter time than the other four treatments.

Table 4 : Mean Pathogenic Index of chickens under each treatment

Treatment	Mean	N	Std. Deviation
Ocular	0.18	20	.554
Water	0.74	20	1.122
Feed	1.35	20	1.105
Spray	0.42	20	.860
Naïve	2.43	20	.233
Total	1.02	100	1.159

Chickens in all treatments have significantly lower pathogenic index than that of chicken in control group. The pathogenic index is not significantly different between birds' vaccinated using spray, water and ocular route of vaccination but it is significantly lower in chicken vaccinated with barley (Table. 18)

The pathogenic index of the challenge virus in the four treatment groups is not significantly different in the two breeds of chicken. This shows that the breed effect on the pathogencity of the disease is not significant.

c) Survival rate of chicken after challenge

The result from this study revealed that the survival rate of the chicken after wild ND viral challenge was higher in all treatments than the control group, but between breed difference in the survival of the field challenge is not significantly different (P=0.6).

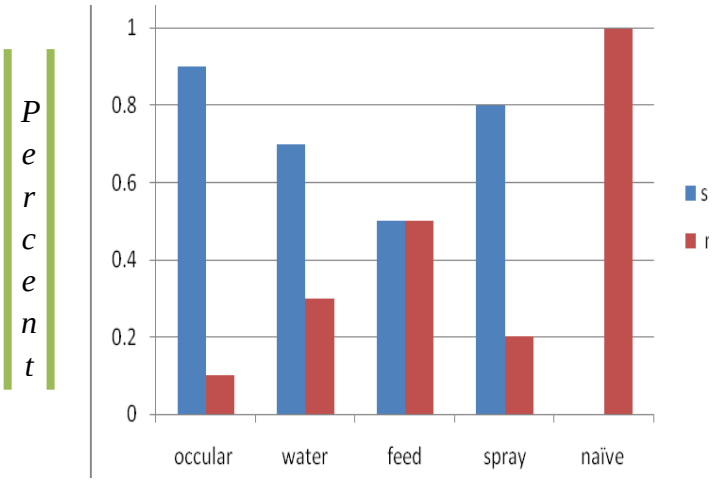

Fig. 1 : Post challenge percent survival of chicken in the five treatment groups (blue= survive & Red= dead)

Survival probability of chicken in different treatments groups is measured using mortality probability of chicken with relation to their HI titter.

d) Relationship between mean HI titer and mortality of experimental chickens

The picture below shows the mean titter of HI for the four treatments with relation to the control group is high. The result shows that the loss related with Newcastle disease in unprotected flock was up to 100%.

Table 5 : Pathogenic index difference of different treatments

Rx	Rx	M D	S. E	Sig.	95% C I	
					LB	UB
water	Eye	.559	.268	.234	-.19	1.30
Feed	Eye	1.167*	.268	.000	.42	1.91
	Water	.609	.268	.163	-.14	1.35
Spray	Eye	.236	.268	.903	-.51	.98
	Water	-.322	.268	.748	-1.07	.42
	Feed	-.931*	.268	.007	-1.68	-.19
Control	Eye	2.250*	.268	.000	1.51	2.99

Water	1.691*	.268	.000	.95	2.44
Feed	1.083*	.268	.001	.34	1.83
Spray	2.014*	.268	.000	1.27	2.76

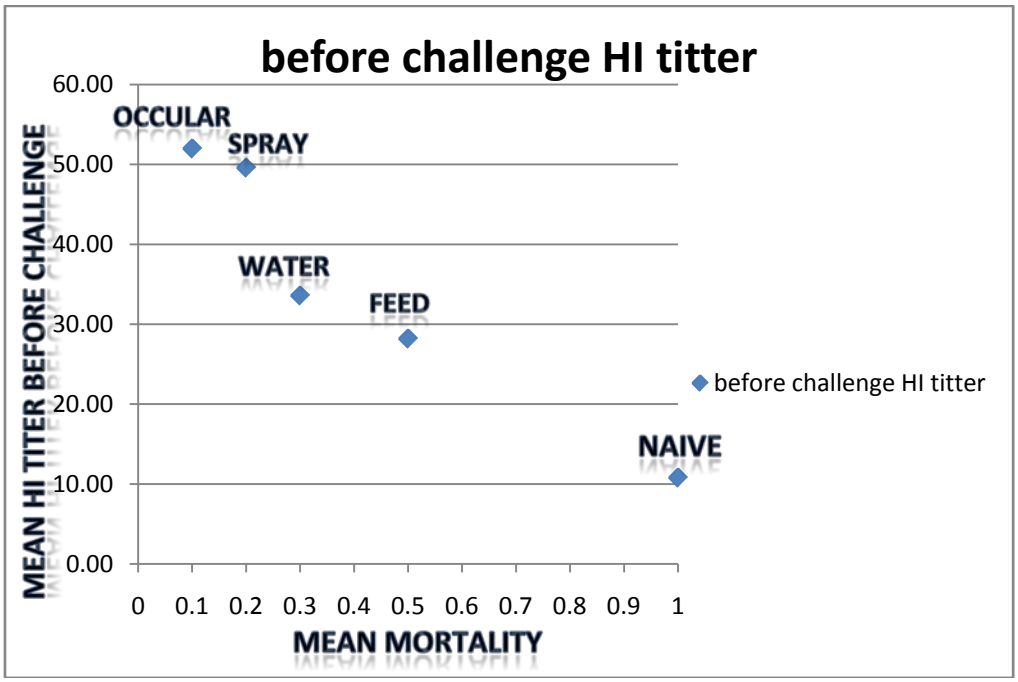

Fig. 2 : Mean HI titter before the challenge

The survival time of chicken after the challenge is lower in control group than any other treatments. The survival time significantly higher in chicken in chickens under ocular and spray route of vaccination. Survival rate of chickens which takes vaccine through vaccine treated feed is relatively lower than other treatment groups.

The survival curve showed that more than 75% of the chicken in the ocular, spray and water treatment groups survived the mortality. The survival rate of chicken in the control treatment was zero percent after 8 days post challenge (Figure 3).

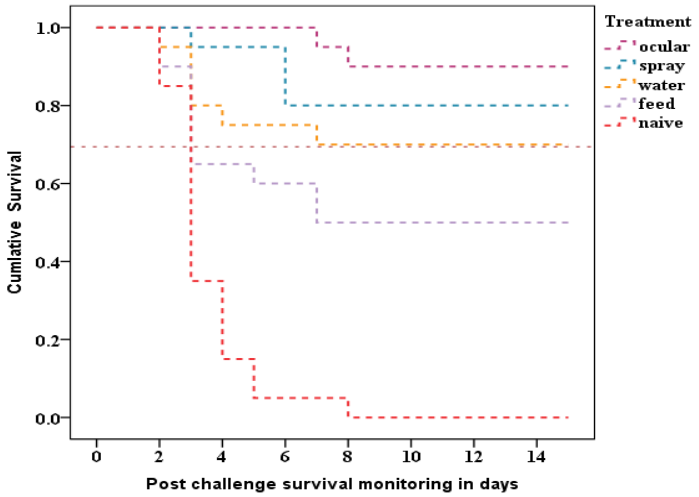

Fig. 3 : Post challenge survivality of chicken

IV. Discussion

The protection level of the vaccine in the naive treatment groups in this study was in agreement with Musa *et al.* (2010) as the HI titter of chickens that were not taking no vaccination have unprotected antibody titter. The high mortality of chicken in naive group had similarity trend with control groups (naïves) of other

works and literatures elsewhere (OIE, 2013), (Hussain *et al.,* 1988) and (Nasser *et al.,* 2010). According to the FAO, 2005 and manual by OIE, 2013; NDV can cause 100% mortality in unprotected flock devastating outbreak condition.

In study which performed using bran, ground grain and water as a vehicle by (Abdu *et.al,* 2012); water vaccination was more protective than vaccination using feed as a channel. The difference in the immune response of chicken on vaccinated with water and feed is the time taken to take the formulated vaccine. It is taking longer in feed channel than that of water. This is mainly related with inadaptability of the chicken for the feed that the vaccine is constituted. It is believed that prior adaptation for the grain in which that vaccine was given may be increase the efficiency of the vaccine. Study by Musa *et al,* 2010; the mortality of chicken that were vaccinated with vaccine treated sorghum is devastating (up to 100% mortality), this is different from the result of the current study. The finding of this study on treated barley is different from the findings of Nasser *et al.,* (2010) which report more than 90% protection. This might be due to the number of animal under the challenge and the difference in the type of chicken used in the treatment. Broiler chicken was used by Nasser *et al.,* (2010) and according to Mozaffor *et al.,* (2010) broiler chicken have higher sero conversion for Newcastle disease than that of layer chickens.

In this investigation, better results were obtained when chicks were vaccinated via eye drop and litter spray route. This agreed with the findings of vaccination trials conducted in other African countries, using the same or other thermostable vaccines of ND (Musa *et al,* 2010; Hussain *et al.,* 1988, Foster *et al,* 1997, Khalafall *et al.,* 2004). On the other hand, chicks vaccinated by water showed remarkably lower immune responses and protection rates as compared to ocular and spray vaccination, but higher than that vaccinated with feed as a channel. The reduced response of the birds to vaccines that are given by oral routes is mainly due to virus viability be lost at the gastrointestinal tract (GIT), unless high amount of NDV is contained in the vaccine (Shuaib *et al.,* 1985). It is also reported on Spradbrow (1992) that the viral load excreted from orally vaccinated chicken was little or zero after the second vaccination when faecal extracts possessed neutralizing activity, probably associated with IgA antibody.

Based on these findings, the intra ocular rote administration of I2 vaccine is recommended for the vaccine application especially for village chickens where number of chickens in a flock is small. However, to implement conventional vaccination methods chickens are difficult to catch which is also reported by Latif *et.al.,* (1992). But spray vaccination which can be performed by middle level professional easily is a simple means of vaccinating chicken. Following the virus administered by spray it follows the natural route of infection, it reaches the upper respiratory tract through the naso-lacrymal duct where it multiplies to induce the required immune responses. This technique can also be practical on commercial production system that has large numbers of chickens to be immunized all at once.

V. Conclusions and Recommendation

ND is responsible for massive rural chicken loss that makes farmers to loss their trust in poultry production as a means to alleviate poverty and improve family nutrition. The current experimental ND vaccination trial of this study provides an alternative vaccine administration routes. This will contribute in researches and targets towards prevention and control of ND disease. This is believed to have a potential for significant improvement in the livelihood of poor people. Accordingly, protection level of intraocular and spray vaccination is better than that of water and feed vaccination. However, the litter spray route is the easiest, affordable and highly effective means of vaccinating village chickens.

Based on the above conclusion the following recommendations were forwarded: Newcastle disease prevention and control with routine vaccination program should be of the first priority in village production system. Among the vaccination routes tested in this experiment, litter spray vaccination of thermo-stable vaccine is the preferable one for scavenging small scale production system where ocular vaccination application is very difficult. In addition for proper vaccination of chickens, training on management practices; village bio-security and nutrition must be implemented with. To complete the output of this result, on farm evaluation of ND vaccination and training of farmers in the study area should be implemented and the effect of the intervention should be quantified.

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