

*Full Length Research Paper*

# Investigating the Safety and Efficacy of a Combined Vaccine Approach for Newcastle Disease, Fowl Pox, and Fowl Typhoid in Controlled Laboratory Conditions

Hana Tadesse<sup>1\*</sup>, Senait Belete<sup>2</sup> and Benti Deressa<sup>3</sup>

<sup>1</sup>School of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia.

<sup>2</sup>National Veterinary Institute (NVI), Debre Zeit, Ethiopia.

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An experimental study was conducted on day-old chicks to evaluate the safety and efficacy of combined Newcastle diseases (ND), fowl pox (FP) and fowl typhoid (FT) vaccine. The vaccine was prepared using the Lasota strain of Newcastle disease virus (NDV), the FP strain of fowl pox virus (FPV) and the 9R strain of FT. The vaccine was found safe, as no clinical signs or mortalities were observed. Post vaccination Haemagglutination Inhibition (HI) titre for ND was above the required protection level ( $\geq 1:16$ ) and its geometric means (GM) were 0.0098, 0.0063 and 0.0059 for group one, two and three respectively, who received conventional and combined vaccine. The difference in GM between the three vaccinated groups were not significant ( $p=0.544$ ). The trivalent combined vaccine did not show significant difference in the HI titre result among the groups that were given conventional vaccines and the other two experimental groups which received trivalent vaccine ( $p=0.257$ ). From 75 samples, 73 (97.3%) were positive for FT through rapid slide agglutination test (RSAT). The chicks were challenged separately for the three diseases using the specific pathogens. Both combined and conventional vaccine conferred protection upon challenge. For ND challenge, 93.3% ( $n=14/15$ ) of the control groups died. From FT and FP control groups 86.6% ( $n=13/15$ ) and 20% ( $n=3/15$ ) respectively died up on challenge. Both combined and conventional vaccine type conferred a similar and good level of protection. However, the use of combined vaccine has considerable advantage particularly in terms of convenience and cost effectiveness to control multiple diseases through simple immunization schedule. Further studies were recommended on the development of combined avian vaccines in Ethiopia.

**Key words:** Newcastle disease, fowl pox, fowl typhoid, experimental study, combined vaccine.

## INTRODUCTION

Infectious and non-infectious diseases are major threat to human and animal life throughout the globe. The number

of people and animals dying due to infections are far greater than any other reason in the world every year. With

\*Corresponding author. E-mail: [tadessehanna@gmail.com](mailto:tadessehanna@gmail.com).

increasing industrialization and intensification of rearing systems, the disease pattern in domestic fowl is changing. There are increasing problems and thus increased risk of disease entry to local chicken by movement of infected birds or contaminated products or materials from other places (Desalew, 2012).

The major endemic diseases, which constitute major constraints to poultry production in Ethiopia, include Newcastle disease caused by virulent strains of avian Paramyxovirus type 1 (APMV-1) serotype of the genus Avulavirus belonging to the subfamily *Paramyxovirinae*, family *Paramyxoviridae*, Fowl pox caused by Avian poxvirus, and Fowl typhoid caused by the highly pathogenic chicken-adapted *S. enterica* biotype Gallinarum. These diseases are prevalent in different parts of Ethiopia and pose significant economic problems to poultry production (Hailu, 2012).

In all countries where those diseases occur, vaccination is accepted as the method of control for the prevention of the diseases expansion (Petra and Karen, 2012). In Ethiopia, individual or separate vaccination program of different livestock diseases has been practiced for years (Gelagay et al., 2012). The individual vaccination strategies has several constraints like stress during individual handling, vaccination cost, number of inoculations, compliance to the vaccine schedule and logistic costs.

In order to increase the probability of early control and/or eradication of the most important diseases of poultry at national level and reach the target vaccination coverage, the production of combined vaccine is very advantageous both from the technical and economic point of view. Therefore, the objectives of the present study was to compare antibody production level of national veterinary institute (NVI) produced Newcastle disease, fowl pox and fowl typhoid conventional vaccines with the experimental combined vaccine and to evaluate the safety and efficacy of newly produced combined vaccine under laboratory condition.

## MATERIALS AND METHODS

### Experimental animals

A total of SPF 135 white leghorn day-old chicks were raised under intensive management system and used in all the vaccination experiments. The chicks' house was fumigated with formalin before the introduction of chicks and bedded with disinfected wood shavings.

### Experimental design

Randomized controlled design was used. Experimental chicks were randomly placed in four vaccine groups ( $n= 30$  chicks /group) (Table 1), identified by leg band. Forty-five chicks were used as a control for the three treatment groups ( $n= 15$  control chicks /group).

### Master seed management

All vaccine seed strains were supplied by African Union Pan African Veterinary Vaccine Center (AU-PANVAC). All the vaccine seed

strains were live-attenuated (Lasota, FPV and *Salmonella Gallinarum* 9R).

### Production of experimental combined vaccine

ND ( $10^7$  EID<sub>50</sub>/ml) and FP ( $10^3$  TCID<sub>50</sub>/ml) vaccines were prepared using specific pathogen free eggs (SPF) while FT vaccine was produced by using *Staphylococcus gallinarum* 9R strain on *S. Gallinarum* broth ( $5 \times 10^7$  CFU) medium (World Organization for Animal Health (OIE), 2015). The vaccines were produced by mixing one part of fowl pox virus (FPV) (500 ml), and two parts of each Newcastle disease virus (NDV), (1000 ml) and FT (1000 ml). Likewise, 2500 ml of freeze-drying media (Lactalbumin Hydrolysate 5% and sucrose 10%) were prepared before mixing. Totally, 5000 ml solution was mixed and dispensed into sterile glass vials with 2.5 ml quantities per ampoule by using a sterile calibrated automatic syringe. The titer was expressed on the bases of embryo mortality for ND, cytopathic effect for FP and culture turbidity for FT; and calculated by spearman-Karber method (Kiril et al., 2017). The safety, titration and the sterility of each vaccine was checked separately before combining according to World Organization for Animal Health (OIE) (2015).

### Validation of the vaccine

After lyophilization, each vial was checked for vacuum with vacuum tester. In addition, freeze-dried vaccine was checked for sterility and titration according to World Organization for Animal Health (OIE) (2015)

### Safety test

The safety test was carried out by using 10 seven day-old chicks, each of them was inoculated through eye-drop, wing web, and subcutaneous routes of inoculation with single dose of the combined vaccine. After inoculation, they were clinically checked for 3 weeks to determine the presence of local and/or systemic adverse reactions, which may develop after vaccination. Moreover, 5 six week-old chicks received 10 doses of the combined vaccines by the same route and observed for 3 weeks (World Organization for Animal Health (OIE), 2015).

### Serum collection

The blood samples were collected randomly from experimental groups of chicks to assess the immunity level prior to vaccination and after vaccination at day 21. The blood samples were collected from different groups of chicks from their wing vein by using 3ml sterile syringe. After collection, the syringe was kept in slanting position over-night in order to collect the serum samples, the sera were collected and inactivated in water bath at 56°C for 30 min. After inactivation, the sera samples were tested, to determine the antibody level by using hemagglutination inhibition test for Newcastle disease and rapid slide agglutination test for fowl typhoid (World Organization for Animal Health (OIE), 2015)

### Experimental grouping

The chicks in group one were vaccinated with HB1 and Lasota conventional vaccines at 7 days and 5 weeks of age for Newcastle diseases (ND) through eye drop; at 9 weeks of age FP vaccine through wing web and, at 10 weeks of age with FT vaccine through subcutaneous route (World Organization for Animal Health (OIE), 2015) Group two and three were vaccinated two times with combined

**Table 1.** Vaccine type, age, dose and number of chicks within a group.

Group	Vaccination age	Type of vaccine	Dose	Booster	No. of chicks
1	7 days and 5 weeks booster	HBI	10 <sup>7</sup>	1x HBI	30
		Lasota	10 <sup>7</sup>	1xLasota	
	9 weeks	FP	10 <sup>3</sup>	1x FP	
	10 weeks	9R (FT)	10 <sup>7</sup>	1x FT	
2	7 days and 8 weeks booster	Combined	-	2xcombined	30
		Combined	-		
3	14 days, 9 weeks booster	Combined	-	2xcombined	30
		Combined	-		45

vaccine at 1<sup>st</sup> and 2nd vaccination, the chicks of the group two received the vaccine at 7 days of age and boosted at 8 weeks of age; group three received the vaccine at 14 days of age and boosted at 9 weeks of age (Table 1).

#### Challenge pathogens and experiment

Virulent strains of velogenic NDV, FPV and FT were obtained from the NVI Research and Diagnostic laboratory, which is previously collected and confirmed positive for the three diseases, from different regions of Ethiopia during different outbreaks and given to chickens at a titre of 10<sup>6</sup> EID<sub>50</sub>/bird, 10<sup>5</sup> TCID<sub>50</sub>/bird and 10<sup>7</sup> CFU/bird, respectively. The titres of the challenge viruses and bacteria were checked before challenge according to the NVI's standard operating procedure (SOP). The challenge test was conducted separately for ND, FP and FT. Ten chicks (n=10) from each treatment group for each pathogens and five (n=5) from control groups for each pathogens were challenged 5 weeks after the last vaccination and they were kept under strict quarantine. They were observed for disease symptoms and gross pathological lesions for 14 days post-challenge. Detail clinical and post-mortem examination were conducted especially on birds showing clinical signs of those diseases.

#### Statistics

Data collected were entered into micro soft (MS) excel spread sheets and statistical package for social science (SPSS) version 20 was used to analyze the data. Descriptive statistics were used to analyze the variation in mean antibody titer and occurrence of disease following challenge trial among treatment groups. A 5 % absolute precision and 95% confidence interval was used and level of significance was set at  $p=0.05$ .

## RESULTS

#### Safety

In safety trial, birds were euthanized at six weeks post-vaccination; necropsies were performed and samples were taken and tested. No abnormal clinical signs or mortalities were observed either in the group of seven-day old chicks of the safety group, which received one dose of vaccine or in the six week-old birds receiving ten doses at different routes of inoculation of the vaccine.

#### Serological response to the vaccine

For efficacy of the trivalent vaccine, a total of 75 sera samples were randomly collected from vaccinated birds (group one, group two and group three) and the geometric mean HI titre and rapid slide agglutination test (RSAT) were observed for ND and FT, respectively. Fowlpox is confirmed through challenge. In all vaccinated groups of chicks, the HI antibody levels were above the required protection level which is 1:16 for NDV (World Organization for Animal Health (OIE), 2015). The analysis of the data showed that the trivalent vaccine did not show significant difference ( $p=0.544$ ) in the GM HI titre between groups (Table 2). The lowest HI titre was 1:16 (group one and two) and the highest HI titer was 1:2048 (group two and three). The trivalent combined vaccine did not show significant difference ( $p=0.257$ ) in the HI titre result among the groups that were given conventional NVI produced vaccines and the other two experimental groups which received trivalent vaccine. From 75 samples, 73 (97.3%) were positive for FT through rapid slide agglutination test (RSAT) and test results observed among the groups (one, two and three) that were given one group with conventional NVI produced vaccines and two groups experimental trivalent vaccine were not significantly different ( $p=0.618$ ) (Table 3).

#### Response to challenge

The challenge protection produced by vaccinated groups in comparison to the unvaccinated control groups showed that, from 90 birds challenged only 3 birds (3.3%) died and 87 birds (96.7%) survived from vaccinated groups of chicks after challenge with virulent local strains of respective virus and bacteria species (Table 4). From 45 unvaccinated controls challenged, 32 birds died (71.1 %) only 13 birds survived (28.9%); among those survivors 12 of them were FP survivors and showed typical clinical sign of FP (Figure 3). In terms of challenge protection, significant difference ( $P=0.001$ ) was observed among vaccinated groups in comparison to that of unvaccinated control groups. However, there was no significant difference in terms of

**Table 2.** Hemagglutination inhibition titre of NDV.

Variable	GMT	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048
<b>Group 1</b>									
No.		1	3	6	9	5	1	-	-
Percentage	0.0098	4	12	24	36	20	4	-	-
<b>Group 2</b>									
No.		1	3	5	4	5	5	1	1
Percentage	0.0063	4	12	20	16	20	20	4	4
<b>Group 3</b>									
No.		-	3	5	5	6	4	1	1
Percentage	0.0059	-	12	20	20	24	16	4	4

**Table 3.** Rapid slide agglutination test for fowl typhoid (RSAT).

Group	RSAT	
	Negative (%)	Positive
Group 1	1 (4.0)	24 (96.0)
Group 2	1 (4.0)	24 (96.0)
Group 3	0 (0)	25 (100.0)
Total	2 (2.7)	73 (97.3)

**Table 4.** ND, FP and FT, vaccinated and control groups challenge survived and died.

Pathogen	Group 1		Group 2		Group 3	
	Conventional {No. (%)}	Control {No. (%)}	Combined {No. (%)}	Control {No. (%)}	Combined {No. (%)}	Control {No. (%)}
<b>Newcastle disease</b>						
Survived	9(90)	0(0)	9(90)	1(20)	10(100)	0(0)
Died	1(10)	5(100)	1(10)	4(80)	0(0)	5(100)
<b>Fowl Typhoid</b>						
Survived	9(90)	2(40)	10(100)	0(0)	10(100)	0(0)
Died	1(10)	3(60)	0(0)	5(100)	0(0)	5(100)
<b>Fowl Pox</b>						
Survived	10(100)	4(80)	10(100)	5(100)	10(100)	3(60)
Died	0(0)	1(20)	0(0)	0(0)	0(0)	2(40)

post-challenge protection ( $p > 0.05$ ) between NVI produced conventional and trivalent combined vaccine.

## DISCUSSION

The three diseases (ND, FP and FT) are a major problem of poultry industry in Ethiopia (Hailu, 2012). In the present study, the production of improved vaccine was achieved successfully. The experiment showed treatment has no pathogenicity effect on vaccinated chicks. Unvaccinated control group of chicks have shown clinical sign of the disease during challenge experiment (Figures 1, 2, 3). The

outcome of infection and interaction between the two viral and one bacterial strain indicated that there is no potential interference with the replication of the pathogenic strains. Mayahi et al. (2013) showed that the use of polyvalent vaccines combined in the manufacturing laboratory can attenuate the interference between these viruses when compared to vaccines associated just before vaccination.

The trial compared that chicks received the conventional vaccines (group one) and combined vaccine (group two and three); group three provided best antibody response. From the results of the challenge tests, the vaccine was effective without any evidence of interference. The vaccine was found to be safe for seven day-old and 6 week-old



**Figure 1.** Prostration of head and neck of a bird that died from NDV infection.



**Figure 2.** Diarrhoea of a control bird that died from FT infection.

chicks. Zou et al. (2013) reported similar arguments; in their findings the results of the challenge tests and performances of birds showed that all the three vaccine was found to be safe even for one-day old chicks in the first week of vaccination.

Ayala et al. (2016) observed that Lasota is much more immunogenic than the Hitchner B1 and strain V4. A number of researchers have reported that live ND vaccines give better protection and health status than killed

vaccines. The use of live vaccines is preferred for priming the birds as it produces local immunity in the mucosal membrane of the conjunctiva, thus providing immediate protection on subsequent exposure with field virus challenge (Patti et al., 2013; Taebipour et al., 2017). In the present experiment, in all vaccinated groups of chicks, the hemagglutination inhibition antibody levels were above the required protection level which is 1:16; it assure the criterion set by World Organization for Animal Health



**Figure 3.** A lesion on the comb and wattle of a control bird that died from FP virus.

(OIE) (2015).

Furthermore, experience in Ethiopia had shown that Lasota vaccine confers immunity for 6 months when administered at 5 weeks of age. The results of vaccine trials in Ethiopia showed that conventional (HitchnerB1 and Lasota) and the thermo stable ND-12 vaccines give similar antibody response and protection against challenge when given via the ocular and the drinking water route (Mayers et al., 2017). In this study, the HI titre 1:16 was considered protective and it was comparable to the results of previous findings (Ayala et al., 2016; Majid, 2014) that reported birds with HI titers 1:16 were protected against challenge with a virulent strain of NDV.

In the present study, the FP strain of FPV was used. The production of good immunity levels by administration of FP vaccine via wing web was confirmed by means of challenge experiment. From 45 birds challenged, none of 30 birds from the vaccinated group were died but only 3 (20%) birds from the control groups were died and 12 (80%) survived with typical clinical sign of the disease (Figure 3), this result is similar to the findings of Meseko et al. (2012). There is no obvious clinical signs were detected among all vaccinated birds twenty-one days post challenge. As for the control group, typical clinical signs of fowl pox (scabs on comb, wattles and legs) were observed seven days post challenge.

Mortality in highly susceptible chicks exposed to virulent strains of *S. Gallinarum* was limited by SG9R vaccine (Wigley, 2017). Chetan et al. (2014) and Łaniewski et al (2014) showed that a 9R vaccine provided excellent protection and is safe for vaccination of 4 week-old chicks. Indeed, in the present study, birds that received the SG9R strain by subcutaneous route showed no evidence of disease.

An ideal vaccine should promote protection of birds against mucosal and systemic infection by effectively stimulating both immune responses (Revolledo and Ferreira, 2012). In the present study, the strain used for *S. Gallinarum* was 9R. 9R is the rough strain that originated from the smooth strain 9S (Paweł et al., 2014). The 9R strain does not contain the somatic antigen characteristics as the smooth forms of *S. Gallinarum* due to the loss of some lipopolysaccharide. The change in lipopolysaccharide reduced the virulence of the strain (Bérto et al., 2015; Immerseel et al., 2013).

In the study findings, out of 75 samples, 73 (97.3%) were positive for FT through RSAT. The antibody response of birds vaccinated with conventional and combined vaccines was very effective. In addition, there were no statistically significant differences in the protection efficacy or immune responses between group one, two and three. In this study, the best protection was observed in group three. The results indicate that the application of combined experimental vaccine is safe when vaccinated at 10 weeks of age. There were no detected clinical signs of disease or mortality due to the vaccine strain during the monitoring period of the safety trial. This result is similar to the findings of Atul et al. (2012) who tested the safety of the SG9R vaccine when administered via injection.

Vaccination of group one with conventional vaccine; group two and three with combined vaccine showed protection rate against challenge with the wild-type SG observed 14 days after one-dose vaccination. Twenty-nine (96.6%) birds survived among vaccinated groups, while from the control non-vaccinated group, 13(86.6%) birds died and this was the same as the findings of Chetan et al. (2014).

In conclusion the combined trivalent vaccine used in this

study was found to be safe as no abnormal signs or mortality was observed during safety test throughout the monitoring period. There was no statistically significant variation in the efficacy of the vaccine between the three experimental groups which received conventional NVI produced vaccines and the experimental combined vaccine.

The combined vaccine gave a similar level of protection to the conventional one based on HI, RSAT and challenge protection test for FP. Immunized animals remained apparently healthy without any signs of illness after experimental challenge with each of three pathogens. However, the use of combined vaccine can facilitate greater convenience, bring down the cost of vaccination significantly, reduce stress to the animal, and reduce vaccination time and logistic costs.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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