

# Combined usage of rHVT-H5 and Re-6 vaccines towards an effective avian influenza vaccination program for commercial layer chickens

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**Vaccination against highly pathogenic avian influenza virus (HPAIV) of subtype H5N1 is one of the possible means to protect chickens against its outbreak in endemic countries. Early vaccination of layer birds with recombinant vector vaccine based on turkey herpesvirus expressing H5 gene (rHVT-H5) provides longer protection while inactivated vaccine Re-6 needs multiple booster doses to achieve continuous antibody titer. Therefore, this study was carried out to offer a vaccination program using live rHVT-H5 and inactivated Re-6 vaccines in field condition. For this trial, five ISA brown commercial layer chicken farms were raised with 500 birds per farm. Two licensed vaccines- rHVT-H5 and Re-6 in Bangladesh were used for this trial where birds in farms 1 and 2 were administered only rHVT-H5 vaccine at one-day-old, and farms 3 and 4 rHVT-H5 vaccine at one-day-old then boosted with Re-6 vaccine at 25 weeks of age. Farm 5 was not vaccinated and functioned as control. Blood samples were randomly collected from 20 birds in each farm at 3-week interval from 2 weeks old till 65 weeks and harvested serum analyzed by haemagglutination inhibition (HI) test for antibodies against HPAIV H5N1. The titer of haemagglutination inhibiting antibodies against AIV H5N1 on farms 3 and 4 was considerably high and remained up to 65 weeks. However, in farm 1 and 2 there was decline in antibody titer after 56 weeks of age. This study demonstrated that the combined use of rHVT-H5 vaccine at one-day-old and Re-6 at 25 weeks of age enhanced a longer lasting protective antibody against circulating HPAIV H5N1 in commercial layer chickens in Bangladesh.**

**Keywords:** Vaccine, Serology, Avian influenza, Poultry.

## INTRODUCTION

Highly pathogenic avian influenza virus (HPAIV) of subtype H5N1 has been testified as an important cause of disease outbreaks in poultry species in many countries of Asia, Europe, and Africa (1-2). The HPAI H5N1 was first recognized in Bangladesh in February 2007 and faced a massive economic loss of US\$ 454.5 million in 2007 to 2008 (3-4). Since then, 556 cases have been reported from Bangladesh and have become prevalent every year mainly during winter season (5-6). These annual outbreaks have been estimated to cause noteworthy monetary losses in the poultry in the country as a result of high mortalities of birds, cost of medication and decreased poultry yields. In a bid to combat influenza outbreaks in poultry, two vaccines against HPAIV H5N1 namely rHVT-H5 (Vectormune AI, Cevac) a live vaccine and Re-6 (Merial) an inactivated vaccine were officially licensed in Bangladesh in 2013 (6-8). The rHVT-H5 is a recombinant vaccine constructed by inserting Haemagglutinin (HA) gene of clade 2.2 HPAIV H5N1 (A/swan/Hungary/4999/2006) together with a cytomegalovirus promoter into a non-essential growth region of FC126 strain of Herpesvirus of turkey (HVT) (9-10). This viral vector vaccine can multiply in host cells with continuous production of humoral and cell-mediated immunity for longer period (8,11,12). Consequently, rHVT-H5 is capable of producing cross protection against clade variation,

immunity, and required single application only (13-14). On the contrary, the Re-6 vaccine was developed from clade 2.3.2.1 A/H5N1 and required multiple doses (15).

Even though there have been studies to show the combined use of live rHVT-H5 with inactivated Re-5 (Merial)/ Re-5 (Qyh Biotech)/Re-6 (Merial)/Egy-flu 1 vaccine to produce protective and long lasting antibodies against HPAIV H5N1 in Egypt (16). There is no published report on enhanced protection of chickens against HPAIV H5N1 by combined usage of rHVT-H5 and Re-6 vaccines to boost longer antibody production against this virus in chickens in Bangladesh. Therefore, this study was carried out to suggest an effective vaccination routine in poultry against HPAIV H5N1 by using live rHVT-H5 and inactivated Re-6 vaccines for commercial layer chickens in Bangladesh.

## MATERIALS AND METHODS

**Experiment design.** Five ISA brown layer chicken farms with 500 birds per farm were selected in collaboration with poultry farmers and raised for this research. The management practices like feeding, housing, daylight, biosecurity, floor space were same for all farms and located in the same geographic area. The birds were reared from one-day-old to 65 weeks of age. Two vaccines against avian influenza subtype H5N1 that were approved by the Government of Bangladesh namely, rHVT-H5 (Cevac) and Re-6 (Merial) were selected for this trial. The farmsteads were divided into five groups as Farm 1, 2, 3, 4, and 5. Only rHVT-H5 vaccine was given to Farms 1 and 2 at one-day-old chickens. In Farms 3 and 4, rHVT-H5 vaccine was used at one-day-old and then Re-6 vaccine used at 25 weeks old chickens. Farm 5 was non-vaccinated (control) and data were used to compare with vaccinated group. Doses and routes of vaccines were 0.5ml subcutaneously for rHVT-H5

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and 0.5ml intramuscularly for Re-6 vaccines.

**Sample collection and preparation.** Twenty blood samples were collected randomly from each farm at 3 weeks interval from 2 to 65 weeks old (22 times). About 1 ml of blood was collected from each bird using sterile needle and syringe from either the jugular vein (one-day-old birds) or wing vein (older birds). Sera were obtained from the clotted blood and stored at -20 °C until required for haemagglutination inhibition test.

**Haemagglutination inhibition (HI) test.** The haemagglutination inhibition (HI) test was implemented on all serum samples using 4 HA haemagglutination units of the antigen according to the OIE guideline (5). Inactivated HPAIV H5N1 antigen and positive control antisera were used for HI test. Geometric mean titer (GMT) was calculated using Microsoft Excel spreadsheet (Microsoft Corporation, USA).

## RESULTS

The maternal antibody against circulating avian influenza virus (AIV) of experimental flocks was absent. The correlation of antibody titer against HPAIV H5N1 virus between Farm 1 and Farm 2 was 0.967 and between Farm 3 and Farm 4 was 0.918. The onset of immunity in all four farms started from 2<sup>nd</sup> week of age and crossed target HI titer of 6 at 14 to 17

poultry either by death or culling in many countries as well as infections and demise in human (17).

In 2013, a recombinant live vaccine rHVT-H5 (Vectorimmune AI, Cevac) has been first licensed in the USA and other Avian Influenza virus (AIV) endemic countries like Bangladesh, Egypt and Mexico for AIV H5N1 (6, 18). At the same time, Bangladesh has been accredited one more killed vaccine Re-6 (Merial). The aim of the trial was to evaluate the antibody titer of rHVT-H5 alone and combination of rHVT-H5 with Re-6 for determining the best vaccination program in field practice.

In the vaccine trialed farm onset of antibody production started between 2<sup>nd</sup> and 3<sup>rd</sup> week of rHVT-H5 vaccination when maternally derived antibody was not appeared. The results comply with the trial of De Vriese *et al.* (19) and they demonstrated the commencement of antibody titer was found to start at 2 to 3 week after rHVT-H5 vaccination in the USA.

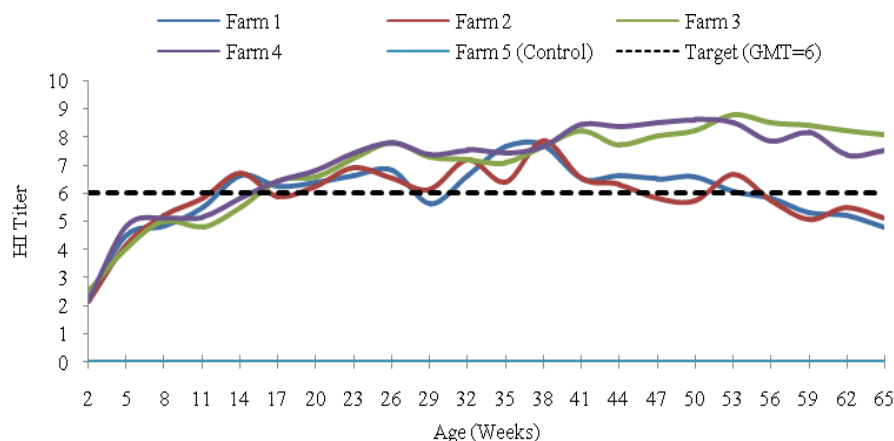


Figure 1: Monitoring of haemagglutination inhibition (HI) titer to vaccination with rHVT-H5 and Re-6 vaccines in commercial ISA brown layer from 2 to 65 weeks of age where rHVT-H5 vaccine was used in Farm 1 and 2, rHVT-H5 along with Re-6 vaccine were used in Farm 3 and 4, and Farm 5 was non-vaccinated control.

weeks of age. The HI titer of both Farm 1 and Farm 2 were found to cross target HI titer 6 at 14 weeks of age with the highest HI titer presented during 38 weeks of age as 7.71 and 7.86, respectively (Figure 1). But the HI titer falls below target HI titer of 6 after 56 weeks of age in both farms. It was also noticed that high titer has been declined at 29 weeks of age in Farm 1. Farm 2 touched target line too during 29 weeks of age that was considered underneath the protection level. The HI titer increased above target titer of 6 after 14 weeks of age and was sustained over the target HI titer of 6 significantly ( $p < 0.05$ ) till 65 weeks of age in both Farms 3 and 4. The highest HI titer in both farms reached during 53 weeks of age as 8.79 and 8.45, correspondingly.

## DISCUSSION

Pathogenic avian influenza subtype H5N1 has been reported to cause major veterinary and public health catastrophe, resulting in severe effects on food security and livelihood due to huge loss of millions of

The vaccination with rHVT-H5 either *in vivo* or to one-day-old age chicks at hatchery can elicit strong immunity by both humoral and cell-mediated immunity (20, 21). Therefore, antibody against AIV persisted for a longer period, sometimes lifelong in infected chickens. HVT is known to persist and replicate in live-cell continually, so that antibody expression of the inserted HA gene inside rHVT-H5 vaccine is likely to replicate endlessly (18, 22). In the present trial, HI titer was identified until 65 weeks of chicken and Palya *et al.* (2018) (23) described that it can persist up to 72 weeks of age in vaccinated chickens. The vaccination with rHVT-H5 has imperative effects of its cross-clade protection properties that play a real remedy against continuous and wide antigenic variation of field AIVs (13, 15).

In contrast, Re-6 is an inactivated vaccine that carry the HA and NA genes from clade 2.3.2.1 H5N1 virus (A/duck/Guangdong/S1322/10 (DK/GD/S1322/10) is widely used in chickens in China, Korea, and Southeast Asian countries including Bangladesh since 2012 (6, 15). There are two clades of AIV H5N1

which have been circulating in Bangladesh since 2012 including clade 2.3.2.1 and clade 2.3.2.1a (7-8). So, Re-6 could be an effective vaccine in Bangladesh though vaccine challenge study is required to be conducted. The Re-6 vaccine required multiple boosters at 2, 7, 12, and 20 weeks of age that is sometimes stressful for birds (1). In the present study, use of Re-6 at 25<sup>th</sup> weeks along with use of rHVT-H5 at one-day-old bird showed protective HI titer and titer persisted over protection line till 65 weeks of age. This result showed agreement with another previous report (16), rHVT-H5 alone or combined with three inactivated vaccines like Re-5 vaccine (Meril), Re-5 vaccine (Qyh Biotech) and Egy-flu 1 vaccine in Egypt during 2014. They stated a combination of live rHVT-H5 and inactivated Egy-flu 1 (bears AIV H5N1) was best vaccine compared to single vaccine of rHVT-H5. The results also showed concordance with the experiment of other researchers (22) and they delineated the use of rHVT-H5 at first day of age and boosting vaccination by inactivated H5N1 after 10 days which gave better protection and HI titer compared to unvaccinated birds or vaccinated by rHVT-H5 alone in broiler chickens (23-25).

Additionally, for the better protection from field AIV H5N1, combination of rHVT-H5 at one-day-age and Re-6 at 25<sup>th</sup> weeks of age can be suggested for vaccination during commercial layer chicken production.

## CONCLUSION

The use of rHVT-H5 at first day of age and followed by boosting vaccination after 25 weeks of age by inactivated Re-6 vaccine which gave better protection compared to vaccinated by rHVT-H5 or Re-6 vaccination alone in commercial layer chickens.

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