



## Research Article

# Evaluation of Quality Control Aspects of Commercially Available Avian Influenza Vaccines in Pakistan

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### ABSTRACT

The present study was conducted to evaluate four (04) commercially available (oil based (n=1) and aqueous (n=3) avian influenza vaccines in Pakistan. The vaccines were tested for sterility, safety, inactivation, potency and residual formalin. All the vaccines fulfilled safety and inactivation tests. Residual formalin ranged from 0.12 to 2.89mg/ml against the recommended level of 0.74mg/ml so only one vaccine (locally produced) had a higher quantity of residual formalin than the recommended level. Potency test for vaccine was performed in hundred broiler chicks, comprising of fifteen birds in each group. Locally produced vaccines were found to elicit humeral immune response earlier as compared to the imported vaccine. It was concluded that primary vaccination with locally produced vaccine and booster vaccination with imported vaccine may help in earlier immunization and persistence of antibodies for longer time.

**Key Words:** Avian influenza, Vaccines, Sterility, Safety, Potency, Inactivation, Broiler, Formalin

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### INTRODUCTION

Avian influenza (AI) is a viral disease of poultry which was first diagnosed in Pakistan in 1994. The disease caused by serotype H7N3 produced high mortality among the affected flocks especially in the broiler breeder rearing areas of the country (Muneer *et al.*, 1995). An influenza outbreak in northern areas of Pakistan was reported in 1999, which resulted in 10–20% mortality with decrease egg production from 10 to 75%. It was found to be H9N2 subtype and was named as A/Chicken/Pakistan/3/99(H9N2) (Naeem *et al.*, 1999). Avian influenza due to H<sub>5</sub>N<sub>1</sub> was first detected in two poultry farms at Charsadda and Abbottabad in Northern areas of the country in February, 2006 (Muhammad, 2006).

Avian influenza (AI) caused heavy economic losses resulting from high mortality and extremely low productivity in chickens in different parts of the country during 2003–04. The spread of AI has been reported through water fowls, shore birds, wild birds and chickens as well, three serotypes of avian influenza i.e. H<sub>5</sub>, H<sub>7</sub> and H<sub>9</sub> have been isolated from poultry from various parts of the country (Saeed *et al.*, 2012; Naeem *et al.*, 2007; Naeem and Jalali, 2005).

The virus belonging to each of the above types could either be highly pathogenic or of the mild nature. Viruses in this group have the ability to change their pathogenicity as a result of mutation. In this way a low pathogenic form may change to a highly pathogenic form and vice versa by different mechanisms (Perdue and suarez, 2000). Use of subtype specific vaccine can be helpful in inducing protection against the prevailing strain of avian influenza

virus (Swayne and Halvorson, 2003; Naeem and Jalali, 2005).

Vaccinated flocks cannot be considered influenza virus free but vaccination typically reduces the amount of virus shed in experimentally vaccinated challenge birds thereby reducing virus shedding and potential transmission of the virus to other birds (Halvorson *et al.*, 1987). The present study covers the quality control evaluation of four commercially available AI vaccines in Pakistan.

### MATERIALS AND METHODS

A total of four vaccines, being marketed in Pakistan, comprising three locally produced and one imported were evaluated in the present study. The vaccine coding and all relevant details are available in table 1.

Table 1: Details of AI vaccines being used in the present study

Vaccines code	Source of the vaccine	Type of adjuvant	Serotypes present
VQC-58	Local	Aqueous	H9
VQC-59	Local	Aqueous	H5
VQC-60	Imported	Oil emulsion	H5
VQC-61	Local	Aqueous	H5+H9

### Sterility Test

The test was performed as per procedures described in OIE manual (2013a), three plates of each media (Brain Heart Infusion, Reinforced Clostridium, Sabaroud & MacConkey agars) were used. After confirming sterility of media prepared, 200ul of the vaccine sample was spread out on to the surface in each of two media plates and incubated

Table 2: Pre and post vaccination Geometric Mean Titers (Log<sub>2</sub> GMT) of avian influenza H5&H9 antibodies in serum samples of broiler chickens

Vaccine samples tested	Titer at day 0	Log <sub>2</sub> GMT Titer at day 15 <sup>th</sup> post-vaccination	Log <sub>2</sub> GMT Titer on day 30 <sup>th</sup> post-vaccination
VQC-58	00	9.2	8.25
VQC-59	00	7.75	7.9
VQC-60	00	3.5	6.75
VQC-61	00	10.0	7.0
Control	00	00	00

initially for 24 hours at 37 °C except for Sabaroud Agar, which was incubated at 25 °C and then observed daily till 07 days.

#### Safety Test

Double dose of each vaccine sample was injected in each of five birds (three week old disease-free and un-vaccinated) intramuscularly at thigh region and were observed for 21 days for any clinical sign and mortality (OIE, 2013b). The birds were slaughtered for postmortem on day 21 post-vaccination and observed for any lesions at internal organs and as well as at the site of injection.

#### Inactivation Test

It was performed by inoculation of three chicken embryonated eggs (9 to 11 days old) per vaccine and was incubated for 72 hours. Amnio-allantoic fluid from dead and surviving embryos was tested for Hemagglutination (HA) activity (King, 1991).

#### Potency Test

Hundred day-old broiler chicks were reared at animal house of NVL, Islamabad under standard husbandry conditions. At the age of day 7, blood samples were taken and chicks were divided into five groups i.e. A, B, C, D and E having fifteen birds in each group. Groups A, B, C and D were vaccinated with four different commercially available AI vaccines, VQC-58, VQC-59, VQC-61 and VQC-60, respectively. Group E was kept as unvaccinated control.

Route and dose of vaccination was adopted according to the manufacturer's instructions given on vaccine bottles. Blood samples were again collected on days 15 and 30 post-vaccination. Sera were separated by centrifugation the clotted blood samples at 1500 rpm for 15 minutes and preserved at -20° C. In order to monitor the antibody titers, Haemagglutination (HA) and Haemagglutination Inhibition (HI) tests were performed (Olsen *et al.*, 2003). The results of antibody titers were calculated as geometric mean titers (GMT) (Brugh, 1978).

#### Formalin Residual Test

0.5 ml of a 1:200 dilution of the vaccine was examined by adding 5 ml of the Methyl Benzo Thiazol-2-one Hydrazone (MBTH) reagent, and allowed to stand for 60 min. Then 1 ml of Ferric Chloride-Sulphamic acid reagent was added and allowed to stand for 15 min. The absorbance of vaccines and standards was measured on a spectrophotometer at the maximum at 628 nm in cuvettes using the reagent blank as compensation liquid (VICH GL25, 2002; Anonymous, 2002).

## RESULTS

All vaccines passed safety and inactivation test requirements. Vaccines coded as VQC-60 and 61 complied sterility test while VQC-58 and VQC-59 could not. All vaccines were also subjected to potency test. The pre-and post-vaccination geometric mean antibody titers

(Log<sub>2</sub> GMT) are shown in table 2. The VQC-58, VQC-59, VQC-60 & VQC-61 gave the titers of 9.2, 7.75, 3.5 & 10, on day 15 post-vaccination, respectively. The titers 8.25, 7.9, 6.75 & 7 were recorded on day 30 post-vaccination, respectively. The result indicated that the locally produced vaccines gave better titers on day 15<sup>th</sup> in comparison with imported vaccine which illustrated higher titer on day30th (table 2).

Residual formalin was found to be 0.12, 0.14, 0.176 and 2.89 mg/ml in VQC-58, VQC-59, VQC-60 and VQC-61, respectively (table 3).

Table 3: Formalin residual concentration in vaccine samples

Vaccine tested	Formalin residual concentration(mg/ml)
VQC-58	0.12
VQC-59	0.14
VQC-60	0.17
VQC-61	2.89

## DISCUSSION

Avian influenza viruses can cause a wide range of disease in poultry from a sub-clinical infection to a devastating disease with high mortality. Although avian influenza is best controlled by preventing the introduction of virus, vaccines can provide an effective way to contain the disease should the virus is introduced, (Suarez, 2000).

In this study, evaluation of four commercially available avian influenza vaccines was conducted: local vaccines gave the antibodies titer (GMT-9.2, 7.8 & 10) on day 15<sup>th</sup>. Whereas imported vaccine gave a low titer (GMT-3.5) on day 15 post-vaccination. Sultan and Hussein, (2008) also reported a similar data, where they found that, vaccination of broiler chickens with H<sub>5</sub>N<sub>2</sub> and H<sub>5</sub>N<sub>1</sub> oil-emulsion vaccines at 10-days of age gave adequate HI titers 6 to 7.6 and 6.2 respectively. Regarding formalin residual, one vaccine showed higher than standard recommended level of 0.74mg/ml, (Federal register, 2003).

The result indicated that the locally produced vaccines gave better titers on day 15th in comparison with imported vaccine which illustrated higher titer on day30th which is in congruent with Salama *et al.*, (2013) who have reported that oil emulsion vaccine could not produce good titer at day 14th in broiler chickens and titers increased slowly after day 20th.

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