Efficacy evaluation of commercial vaccines against circulating filed isolates of avian influenza H9N2 and infectious bronchitis viruses in broiler chickens

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To the Editor,

One of the primary measures used for prevention of infectious diseases is vaccination. Avian influenza (AI) is a viral disease that is contagious in nature and is caused by avian influenza virus (AIV) (1). Infectious bronchitis (IB) is caused by infectious bronchitis virus (IBV) belonging to genus gammacoronavirus of the *Coronaviridae* family (2). An optimum approach to control a disease and obtain maximum protection is to use the vaccines based on the strain prevalent in the area. Despite continuous vaccination against H9N2 and IBV, outbreaks of H9N2 and IBV are often reported in the vaccinated poultry flocks. This situation has challenged the efficacy of control strategies of poultry diseases. A new debate has started whether the vaccinal strains are different from field strains or whether new strains have emerged as a result of immune pressure (3).

Continuous surveillance is needed as IBV and H9N2 are both RNA viruses and undergo a high rate of mutations; which often render the subsequent viral mutants less antigenic, or non-antigenic compared with previous viral strains, thus undermining any vaccine cross reactivity. In the areas where a local strain is not available for vaccination, heterologous vaccines are more likely to be useful. In this case, using different serotypes of vaccines increases the likelihood that at least one of the strains will prove to be antigenic, and capable of eliciting an effective host immune response (4). The aim of this study was to survey protective effects of most frequently used vaccines against H9N2 and IBV.

MATERIALS AND METHODS

Sixty day-old broiler chicks were obtained from a commercial hatchery and were divided into four different groups (A, B, C and D) with 15 birds per group. Standard rearing and management conditions were followed. *Ad libitum* feed and water were offered to these birds. At the age of 7 days, 0.25 mL H9N2 vaccine (Otto Fluvac) was administered subcutaneously to all birds of group A while group B was kept as positive control for H9; this group

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received only H9 challenge but no H9 vaccine. Similarly, again at 0-day of host age, 0.2 mL IBV vaccine (Gallivac IB 88 and Bioral H120, Merial) and at day 14 the second dose (0.2 mL) of IBV (Bioral H120 and Merial) was administered intranasally to all birds of group C while group D was kept as positive control for IBV as this group received only IBV challenge without vaccine.

Serum antibody titer

At 21 days of age, serum was collected from all birds. Antibody titer against H9N2 of group A and B and titers against IBV of group C and D weere checked by ELISA (IDEXX, USA) according to the guidelines of the manufacturer. S/P ratio was calculated, and a ratio greater or equal to 0.5 was considered positive for H9N2 while ratios greater or equal to 0.2 were considered positive for IBV antibodies, suggesting that the birds were vaccinated.

Challenge experiment

The birds in groups A and B were challenged with H9N2 field isolate according to the recommended EID50 (106.5/0.1 mL/dose) and birds in group C and D were challenged with IBV field isolate according to the recommended EID50 (106/0.1 mL/dose). After challenge, the birds were monitored under close observation for clinical signs/ mortality over a two-week period.

Histopathological examination

On days seven and fourteen post-challenge, five birds from each group (A, B, C and D) were randomly sacrificed, and the trachea, lungs and intestine from birds of groups A and B while trachea, lungs and kidney from the birds of group C and D were collected and preserved in 10 % neutral buffered formalin for processing in the Pathology section of QOL, UVAS, Lahore, Pakistan.

RESULTS

The results of this study showed that effective antibody response was generated when the hosts were vaccinated with Otto Fluvac, Gallivac IB 88 and Bioral H120 vaccines. No mortality or morbidity was observed in groups A and C (vaccinated groups) while the mortality rate observed in group B (H9 challenged group) was 10%, and 60% in group D (IBV challenged group). H9N2 ELISA S/P ratio in H9 vaccinated group A ranged between 0.518-1.618 and IBV ELISA S/P ratio in IBV vaccinated group C ranged between 0.167-0.413. At day 28, the groups of the birds treated with the vaccines showed no gross or microscopic lesions. The tissues collected on day 35 (one-week post infection) showed histological changes. Kidneys of group D hosts that were challenged with filed isolate of IBV showed severe peritubular congestion and renal tubular degeneration (Fig. 1A), while the tracheas of the same group showed sloughing and degeneration of tracheal epithelium and rounding of tracheal epithelial cells (Fig. B). Similarly, the lungs of the birds of group B that were challenged with H9N2 filed strain showed congestion and hemorrhaging in the pulmonary syncytia (Fig. C) and infiltration of inflammatory cells (Fig. 1D).

DISCUSSION

In this study, efficacy of commercially available vaccines against the field isolates of IBV and H9N2 were evaluated. Fluvac, an inactivated vaccine, was used against H9N2; Gallivac IB 88, a live attenuated vaccine of 793B IBV variant and Bioral H120, a live attenuated vaccine of H120 strain, were used against IB. These vaccines are the most commonly used in Pakistan and have been shown to provide more than 80% immunization against IBV and H9N2. Our finding is in agreement with the study of Terregino et al. 2008 (5). They demonstrated that a single serotype does not provide complete protection against heterologous strains. The use of different types of live IBV vaccines can however provide protection against a wide variety of different heterologous viral strains of IBV. However, many outbreaks of H9 (6) and IBV (7) have been reported in Pakistan in vaccinated flocks. Disease outbreaks among vaccinated host populations may be due to different efficacies of vaccine under field and experimental conditions (8). Apart from these, there are many other factors to be considered regarding

Table I. *ELISA results of Group A, B (H9N2) and C, D (IBV)*

Sample ID	H9N2 ELISA titer results (S/P ratio)		IBV ELISA titer results (S/P ratio)	
	Vaccinated group (Group A)	Control group (Group B)	Vaccinated group (Group C)	Control group (Group D)
1	0.531	0.238	0.236	0.011
2	0.612	0.169	0.301	0.004
3	0.579	0.018	0.214	0.018
4	0.644	0.171	0.205	0.019
5	0.518	0.250	0.273	0.009
6	0.599	0.301	0.367	0.019
7	1.235	0.246	0.413	0.003
8	0.768	0.118	0.189	0.129
9	1.029	0.289	0.255	0.008
10	0.581	0.098	0.167	0.001
11	1.618	0.173	0.311	0.005
12	0.671	0.013	0.257	0.013
13	0.817	0.145	0.289	0.013
14	0.649	0.212	0.311	0.014
15	0.538	0.311	0.291	0.011
Mean	**0.759**	**0.183**	**0.272**	**0.017**

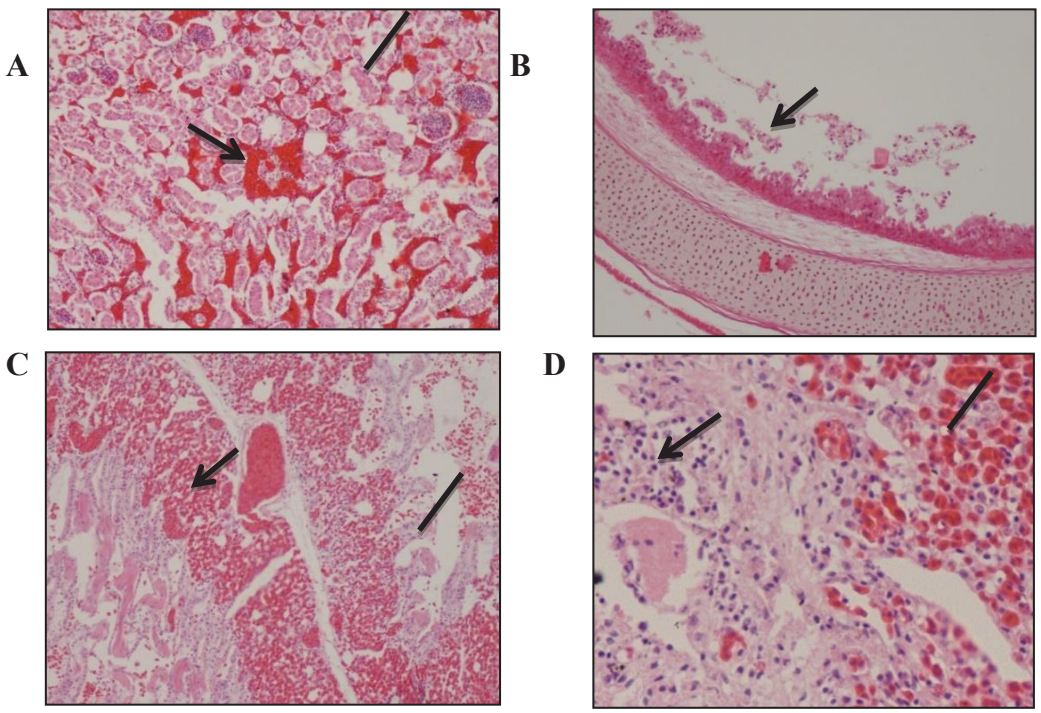

Fig. 1. *Various micrographs depicting pathological lesions.* ***A)*** *Kidney from a group D bird showing severe peritubular congestion, renal tubular degeneration at one week (35th day) post-infection (H and E staining, 10X).* ***B)*** *Trachea from a group D bird showing sloughing and degeneration of tracheal epithelium and rounding of tracheal epithelial cells at one week (35th day) post-infection (H and E staining, 10X).* ***C)*** *Lungs from a group B bird showing congestion and hemorrhages in the pulmonary syncitia at one week (35th day) post-infection (H and E staining, 10X).* ***D)*** *Lungs from a group B bird showing hemorrhage and infiltration of inflammatory cells at one week (35th day) post-infection (H and E staining, 10X).*

lack of vaccine efficacy. These may be infectious, non-infectious or both. Among non-infectious factors are high ammonia levels, high levels of mycotoxins in the feed, poor feed intake. Many infectious factors are immunosuppressive diseases like IBD, Marek's disease and chicken infectious anemia which can affect both types of immunities (innate and acquired) (9). This study is in complete agreement with the results of Shaukal et al. 2016 (10) who reported high efficacy of avian influenza vaccine against prevalent isolates of H9N2 in Pakistan. Macroscopic as well as microscopic changes, such as congestion, hemorrhage and infiltration of inflammatory cells, were observed in kidney, trachea and lungs, and these changes are reported by Zhao et al. 2014 (11).

It is concluded that, despite the reports of genetic mutations and emergence of some new isolates of H9N2 and IBV, the current commercially available vaccines are still effective in providing protective antibody titers under controlled experimental conditions. Poor vaccine protection may be attributed to other environmental or infection-related factors. Furthermore, farm biosecurity needs to be strengthened along with effective vaccination program. Focusing on these will improve the control and prevention of avian diseases including influenza and infectious bronchitis at commercial farms.

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