Bulgarian Journal of Veterinary Medicine (2012), **15**, No 4, 260–272

EFFICACY OF VACCINATION PROGRAMMES USING TWO COMMERCIAL LIVE INFECTIOUS BRONCHITIS VACCINES AGAINST A FIELD IRFIB 32 STRAIN

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Summary

Shirzad, M. R., K. Asasi & A. Mohammadi, 2012. Efficacy of vaccination programmes using two commercial live infectious bronchitis vaccines against a field IRFIB 32 strain. *Bulg. J. Vet. Med.*, **15**, No 4, 260–272.

The effectiveness of two commercial infection bronchitis vaccines including H120 and 4/91 was evaluated in broiler chickens challenged with IBV strain IRFIB32 in different vaccination programmes. For this purpose, 180 day-old Cobb 500 chicks were randomly divided in six treatment groups. Four chicks in each group were humanely sacrificed at 2, 4, 6 and 10 days post challenge for evaluating ciliary activity and molecular detection of the challenged virus in trachea, lung, kidney, testis or oviduct tissues and faeces. A very mild depression and short transient conjunctivitis were observed in challenged vaccinated groups in comparison with unvaccinated challenged chicks. Body weights of the birds in the treatment groups were not significantly different (P>0.05) at the end of the experiment. In ciliostasis test, percentage of protection against chicken groups 2 (4/91, 4/91), 4 (H120/4/91) and 5 (H120, H120) was 85%, 76% and 58%, respectively. All vaccinated challenged chicken groups showed protection against challenge virus, but in the homologue vaccinated birds (4.91, 4.91) protection was more efficient (P<0.05). Antibody titre in H120 vaccinated unchallenged birds (group 1) was lower than in 4.91 vaccinated unchallenged birds (group 6). The birds in groups 4 and 5 had the highest titre of antibody in ELISA (P<0.05) at the end of the study and the difference between these two groups was also significant (P<0.05). The challenged virus were detected in faeces and oviduct or testis tissues up to 10 days post challenge, while the tracheal tissue showed it up to 4 days post challenge. On the basis of viral shedding, vaccination of chicks in groups 2, 4 and 5 provided statistically greater protection than the non-vaccinated control group (P<0.05), 88.75%, 92.5% and 85%, respectively. We conclude that an efficient protection occurred in three different vaccination programmes; however, the birds challenged with homologous virus vaccine resulted in more effective protection.

Key words: broiler, infectious bronchitis virus, Iran, vaccination programmes

INTRODUCTION

Infectious bronchitis (IB), an important respiratory disease of chickens, is characterised by increased oculo-nasal secretion and excess mucus in the trachea accompanied by decreases in weight gain and feed efficiency (Cavanagh & Nagi, 2003). The virus also replicates in the oviduct and testes of infected birds, resulting in

reduced egg production and fertility (Boltz *et al*., 2004). Infectious bronchitis is responsible for severe financial losses to the Iranian poultry industry. Despite the wide use of live attenuated and inactivated vaccines it still remains one of the most important poultry diseases in many countries of the world including Iran (Endo-Munoz & Faragher, 1989; Lambrechts *et al*., 1993). IBV exists as scores of serotypes, cross-protection often being poor. Differences of as little as 5% between the S1 sequences can result in poor cross-protection (Cavanagh, 2003). IBV has a tendency to form new variant strains that are apparently not protected by commercial vaccines. Therefore, vaccination programmes for IBV become difficult to implement because of the challenge of a novel virus and it is of great importance to chicken producers to identify the strains of IBV in their flocks so vaccination programmes can be modified according to the IBV strains present (Hofstad, 1981; Wang *et al.*, 1996).

Since the currently available vaccination programmes do not provide sufficient protection against the field serotype, studying the effectiveness of the vaccine is necessary in selecting an appropriate protocol. Infectious bronchitis virus, an antigenic related to the mass serotype, has been diagnosed in Iran since the early twenties by virus isolation and serological techniques (Aghakhan, 1994). In spite of regular vaccination with live attenuated and inactivated vaccines of mass strains to protect commercial chickens, an epidemiological survey pointed out IB is still responsible for serious financial losses to the poultry industry in the country causing mortality and adverse effects on the quantity and quality of egg production as well as renal failure in broilers and layers. Recently several researchers detected a new serotype (793B strain) by RT-PCR technique in Iranian chicken farms (Nouri, 2003; Seyfiabad Shapouri *et al*., 2004; Shoushtari *et al*., 2009). Momayez *et al*. (2008) studied the cross-immunity of two experimentally oil-emulsion inactivated infectious bronchitis vaccines in Iran. The results showed strong resistance to homologous virus strains. These reports may partially explain the failure of Massachusetts-type vaccines to protect chicken flocks and hence necessitate revising the vaccination programs against IB in Iran. Two vaccinations, a prime and a boost, are given to broilers to induce local antibodies in the upper respiratory tract; the prime is given at 1–3 days of age, and the boost in the field, typically between 12–18 days of age. It remains unclear whether live vaccines including H120 and 4.91 are inefficient in controlling the disease or if vaccination failures are due to inadequate vaccination. This controversial condition has discouraged many farmers from applying IB vaccine.

The objective of this study was to evaluate the effectiveness of two commercial vaccines routinely used against infection bronchitis in different vaccination programmes.

MATERIALS AND METHODS

Experimental design

All experiments were conducted after institutional approval of the animal use committee of Shiraz University. We evaluated the efficiency of two commercial vaccines, live IBV vaccine H120 strain, Massachusetts serotype, (Razi Vaccine and Serum Research Institute, Iran) and live IBV vaccine 4.91 strain, 793/B serotype, (Nobilis Intervet, Holland) in different vaccination protocols. For this purpose, 180 one-day-old broiler chicks,

Table 1. Experimental design including IBV vaccines and vaccination schedules for evaluating the protection against challenge strain IRFIB32

Groups No.	Vaccine strain (day 1)	Vaccine stain (day 14)	Challenge strain IRFIB32 (day 29)
1	H120	H120	No challenged
2	4.91	4.91	Challenged
3	No vaccine	No vaccine	Challenged
4	H120	4.91	Challenged
5	H120	H120	Challenged
6	4.91	4.91	No challenged

Cobb 500, were randomly divided in six treatment groups and reared in separate isolated rooms. The parent flock of the chicks had received H120 and 4.91 vaccine strains, then Massachusetts inactivated vaccine was administrated before coming to lay (20 weeks). The vaccines were diluted according to manufacturer recommendations. Each bird received a commercial dose of vaccine (as 0.1 mL solution) via the oculonasal route. The experimental design is presented in Table 1. The challenge strain, IRFIBV32, showing 95% identity with 793/B serotype had been isolated from a broiler flock that had experienced severe respiratory distress and tracheal petechial hemorrhages in Fars, Iran. The pathogenicity of the isolate was described previously (Boroomand *et al*., 2011). After propagation of the virus in embryonated chicken egg, the virus titre was calculated by semi-quantification assay with Reed and Muench method (Reed & Muench, 1938). At 29 days of age, chickens of groups 2, 3, 4 and 5 were challenged simultaneously with 100 µL challenge virus (IRFIBV32) at a dose of $10^{5.33}$ EID_{50} per 0.1 mL via the oculonasal route. Feed and water were provided *ad libitum*. Birds of all groups were weighed every week. The chicks in all groups were continually monitored throughout the experiment. Signs such as nasal discharge, gasping, coughing, trachea rales, depression, sneezing and sinusitis were recorded for each group. According to previous studies, the scoring for clinical signs and lesions was chosen as +1 to +3 (Avellaneda *et al*., 1994; Wang & Huang, 2000). At 3, 5, 7 and 9 days post challenge, four chickens from each group were humanely euthanatized by cervical dislocation and examined for the presence of gross lesions in trachea (mucin exudates, congestion), lung (congestion, edema), and kidney (swelling, urate deposition). Infectious bronchitis antibody levels were detected by using a commercial enzyme-linked immunosorbent assay (IDEXX ELISA IBV Test kit). Birds were bled at 1 day of age to establish the presence of maternal antibody titres the day before first vaccination, the day before last vaccination and at the end of the experiment.

Assessment of ciliary activity

Four chicks from each group were euthanised on days 3, 5, 7 and 9 post challenge. Trachea were removed aseptically and placed in a Petri dish containing Hanks balanced salt solution (HBSS) to remove mucin out of the tracheal epithelial surface. From each trachea, 10 transverse rings were cut (3 rings from the

upper, 4 from the middle and 3 from the lower part of the trachea). Each of the rings was placed individually in tissue-culture tubes containing 2 mL of Dulbecco's modified eagle medium (DMEM) and then shaking incubated at 37 °C. The tracheal rings were examined for ciliary activity under the inverted microscopy (4× or 10× objective) overnight. The route of ciliary activity scoring is shown in Table 2. A percentage of the ciliostasis score was calculated using the following formula (Cook *et al*., 1996):

$$\left(1-\frac{CS_1}{CS_2}\right)\times 100$$

where: CS_1 – mean ciliostasis score for vaccinated challenged group; CS_2 – mean ciliostasis score for corresponding non-vaccinated challenged group.

PCR detection of the challenging virus

To evaluate the effect of vaccines on the prevention of shedding of the challenged virus, four chicks in each group were humanely euthanised at 2, 4, 6 and 10 days post challenge. RT-PCR test using specific published primer was carried out to detect the challenged virus in the faeces and trachea, lung, kidney and testis or oviduct tissues. We also followed up the presence of vaccine virus genome in different tissues of chicks in groups 1 and 6. The samples were subjected for RNA extraction. Briefly, 1 mL of RNX solution (a commercial RNA extraction kit, CinnaGen, Iran) was added to 50–100 mg of each homogenised sample, then 200 µL of chloroform was added to the mixture. After centrifugation of the samples at 12000 rpm for 15 min, the aqueous phases were transferred to another tube. The RNA was precipitated at 12,000 rpm for 15 min after the addition of an equal volume of isopropanol. The RNA pellet was washed with 75% ethanol, then eluted in 50 µL of distilled water after drying and stored at −70 °C until used.

The extracted RNA was used in reverse transcription (RT) reaction to generate cDNA by use of lyophilised master (Bioneer, Korea). The primer pairs used in the cDNA synthesis and PCR were Dell-R (5'-CAT-TTC-CCT-GGC-GAT-AGA-C-3') and Dell-F (5'-GAG-AGG-AAC-AAT-GCA -CAG-C-3'). For the synthesis of the cDNA, 5 µL of extracted RNA was mixed with 10 pmol of the reverse primer and incubated at 70 °C for 5 min. The contents of each tube were then transferred to the 0.2 mL lyophilised master tubes and the final volumes brought out to 20 µL with diethyl pyrocarbonate water (DEPC). After the addition of 15 µL mineral oil to each tube, the mixture was incubated at 42°C for 1 h, and then incubated at 95°C for 5 min to inactivate the reverse transcriptase. The resultant cDNA was stored at –20 °C.

PCR was carried out in 20 µL volumes containing 2 µL of 10 PCR buffer, 0.2 µL (1 unit) Taq polymerase, 0.6 µL (1.5 mM)

Table 2. Scoring the activity of tracheal cilia as a main index of vaccines protection

Ciliostasis Score	Ciliary Activity
0	100% Ciliary activity, all cilia beating, full protection
1	75% Cilia beating
2	50% Cilia beating
3	25% Cilia beating
4	0% Ciliary activity, non beating cilia, lack of protection

of $MgCl_2$, 0.2 μL (0.1 mM) of dNTPs, 1 μL of each primer (10 pmol), cDNA (4 μL) and 11 μL distilled water (Mardani *et al.*, 2006). The PCR products were electrophoresed on 1% agarose gel in TAE buffer and visualised in a UV transilluminator.

Statistical analysis

The body weight, virus detection, cilia protection percentage, and antibody titre among experimental groups were compared using ANOVA with the non-parametric Duncan test. The P value < 0.05 was considered to be statistically significant. The clinical signs and lesions data were analysed using the chi-square test.

RESULTS

Clinical signs and lesions

Birds of group 3 (unvaccinated challenged) exhibited conjunctivitis, associated with nasal discharge at 24 h after challenge. In addition, the birds appeared lethargic, and in some cases presented with tracheal rales. The period for showing clinical signs was short and the signs disappeared approximately 3 days later. The chicks of groups 2, 4 and 5 showed very mild depression and transient conjunctivitis, however, there were no significant differences between these groups. No clinical signs were observed in chicks of groups 1 and 6. A moderate hyperemia and exudates were seen in the trachea of the chicks of group 3. Mild gross lesions were observed in the trachea and lung of some individual chicks in the groups 2, 4 and 5, however the severity of the lesions was not statistically significant among these groups (P>0.05). Slight hyperaemia and uric acid deposition in kidneys were seen in the individual birds of group 3.

Body weight

At day 6 and 13 post challenge, all the groups showed significantly higher weight than the positive control (P<0.05), how-

Table 3. Mean body weights (g) of chicks in different groups during the experiment (±SD; n=15)

Groups	Age			
	21 days	28 days	35 days	42 days
1 (H120; H120; not challenged)	977[a] ±46.22	1219[a] ±66.38	1652[a] ±57.45	2475[a] ±58.15
2 (4/91; 4/91; challenged)	987[a] ±53.88	1210[a] ±68.47	1646[a] ±68.47	2381[a] ±66.32
3 (not vaccinated; challenged)	940[a] ±50.25	1200[a] ±49.33	1424[b] ±88.52	2070[b] ±86.40
4 (H120; 4/91; challenged)	947[a] ±41.66	1188[a] ±58.73	1610[a] ±80.56	2330[a] ±72.63
5 (H120; H120; challenged)	965[a] ±49.34	1217[a] ±64.19	1638[a] ±61.39	2354[a] ±70.23
6 (4/91; 4/91; not challenged)	910[a] ±58.52	1200[a] ±54.67	1696[a] ±57.41	2381[a] ±62.43

Different superscripts in the same column indicate significant difference (P<0.05).

Table 4. Mean ciliostasis score and protection percentage in chicks vaccinated with different vaccine programmes following challenge with the IRFIBV32 IBV strain at 29 day-old

Groups	Ciliostasis scores*	Protection (%)
1 (H120; H120; not challenged)	0.01	100
2 (4/91; 4/91; challenged)	3.25	85[a]
3 (not vaccinated; challenged)	25.00	**
4 (H120; 4/91; challenged)	6.00	76[a]
5 (H120; H120; challenged)	10.50	58[b]
6 (4/91; 4/91; not challenged)	0.01	100

*mean ciliostasis score/bird for the 10 tracheas examined in each group; maximum possible score (no protection) =40; **positive control.

ever the difference between groups 2, 4 and 5 was not significant (P>0.05). In general, all vaccination programmes protected chickens against the challenge virus (Table 3).

Ciliary activity assessment

The results of ciliary activity are summarised in Table 4. The protection percentages in groups 2, 4 and 5 were 85%, 76% and 58%, respectively and were excellent in contrast to unvaccinated challenged group (group 3). The protection was statistically significant in groups 2 and 4 compared to group 5 (P<0.05). No significant different protection was found between group 2 and 4. In groups 1 and 6 which were not challenged, the protection percentages were nearly 100%.

Antibody responses

The geometric mean of maternal antibody in one day-old chicks was 3100. In chicks of group 5 the highest ELISA titre was 3140 on 42 day-olds, and the lowest titre was 660 on 13 days of age. In contrast to group 3, antibody titres of other groups were low, however, significantly different on 13 days of age (P<0.05). Antibody titres increased in the chicks of all groups, except the unvaccinated challenged group, at 28 days-old. At the end of the experiment (day 42) the chicks of groups 4 and 5 had the highest titre (P<0.05) and the difference between these two groups was also significant (P<0.05). The induction of humoral antibody responses in the chicks of group 5 was later than in the chicks of groups 2 and 4. At all days, the antibody titres of group 6 (4.91, 4.91) was higher than group1 (H120, H120), however, not significantly different. At 42 days of age, the titre of chicks in the positive control group (group 3) was higher than the vaccinated unchallenged groups (groups 1 and 6) but much lower than groups 4 and 5 (Table 5).

Virus detection

The virus detection was performed on 2, 4, 6 and 10 days post challenge as previously described and shown in Table 6. On the basis of the virus detection, vaccination of the chicks in groups 2, 4 and 5 provided statistically greater protection than the non vaccinated challenged

Table 5. Infectious bronchitis ELISA antibody titers following different IB vaccination programmes

Groups	Age				
	13 days	28 days	35 days	39 days	42 days
1 (H120; H120; not challenged)	589 [a]	956 [a]	1400 [a]	1285 [a]	1310[a]
2 (4/91; 4/91; challenged)	660 [a]	1080 [a]	1385 [a]	1639 [a]	1832 [a]
3 (not vaccinated; challenged)	800 [b]	620 [b]	1485 [a]	1531 [a]	1686 [a]
4 (H120; 4/91; challenged)	611 [a]	1028 [a]	1159 [a]	1969 [a]	2479[b]
5 (H120; H120; challenged)	660[a]	980 [a]	1039 [a]	3111 [b]	3140 [c]
6 (4/91; 4/91; not challenged)	532 [a]	1059 [a]	1504 [a]	1458 [a]	1510 [a]

*Different superscripts in the same column indicate significant difference (P<0.05).

group (P<0.05), the protection was 91.25%, 88.75% and 85%, respectively. The samples of kidneys, oviduct or testis and feces were positive for IBV in the chicks of the positive control group on 6 and 10 days post challenge (group 3), however the lung was positive only on 2 days post challenge. The number of positive samples in trachea, lung, kidney, oviduct or testis and feces was 8, 9, 13, 11 and 23, respectively. The highest number of the virus genome detection was seen 2 days post challenge, followed by 4, 6 and 10 days post challenge. It should be noted that the vaccinal viruses were not detected in the chickens of both negative control groups 17 days post vaccination.

DISCUSSION

Increasing the prevalence of different non well cross protective antigenic variant of IBV worldwide, the problem of designing an effective vaccination programme becomes increasingly difficult. It is undesirable to develop a new vaccine for each emerging IB variant, so any effort on finding a vaccination protocol using live-attenuated relevant virus to overcome circulating wild virus is valuable. In this work, the effectiveness of two commercial infection bronchitis vaccines was evaluated in different vaccination programmes. The 4.91 Intervet (Holland) and H120-Razi (Razi VSRI, Iran) vaccines were used because they are the most commonly used vaccines for vaccination in Iranian chicken flocks. The challenge virus, IRFIBV32 strain related to 793/B serotype, has been isolated and well characterised in Fars province, Iran, through an epidemiological survey for detecting new circulating IBV variants involved in regional vaccination failure (Boroomand *et al*., 2011). The time of challenge was chosen according to the natural infection in the field.

Comparison of the clinical signs and lesions of vaccinated and non-vaccinated birds showed that the vaccines induced at least a partial immunity. These findings were similar to those of Gelb *et al*. (2005) and Grgic *et al*. (2009). The clinical signs observed at 2 days post challenge correlated with the virus recovery data and no significant difference was observed among the groups. In the field, husbandry and management conditions are not highly desirable and uniform and in some cases additional viral and bacterial infections

Table 6. IBV detection by RT-PCR in different vaccinated groups following challenge with the IRFIBV32 strain of IBV on 2, 4, 6 and 10 days post inoculation

	Days post inoculation																Total			
	2				4				6				10							
Groups	2[a]	3[b]	4[c]	5[d]	2	3	4	5	2	3	4	5	2	3	4	5	2	3	4	5
Trachea	1/4	3/4	0/4	0/4	0/4	2/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/16	5/16	0/16	2/16
Lung	0/4	4/4	0/4	1/4	1/4	0/4	1/4	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4	1/16	4/16	3/16	1/16
Kidney	1/4	2/4	0/4	1/4	0/4	3/4	0/4	1/4	0/4	2/4	0/4	1/4	0/4	2/4	0/4	0/4	1/16	9/16	0/16	3/16
Oviduct or testis	1/4	2/4	0/4	2/4	0/4	3/4	0/4	0/4	0/4	1/4	1/4	0/4	0/4	1/4	0/4	0/4	1/16	7/16	1/16	2/16
Faeces	1/4	1/4	2/4	1/4	2/4	2/4	2/4	3/4	0/4	4/4	1/4	0/4	0/4	4/4	0/4	0/4	3/16	11/16	5/16	4/16
Total																	7/80	36/80	9/80	12/80
Percentage positive																	8.75	45	11.25	15
Percentage protection																	91.25	55	88.75	85

[a]Group 2: vaccinated at 1 day with 4.91 vaccine, revaccinated by the same strain at 14 days of age; [b]Group 3: challenged unvaccinated birds; [c]Group 4: vaccinated at 1 day with H120 vaccine, revaccinated by 4.91 vaccine at 14 days of age; [d]Group 5: vaccinated at 1 day with H120 vaccine, revaccinated by the same strain at 14 days of age. Values are presented as number of IBV positive chicks /total chicks. The protection is the difference between positive percentage and 100%.

contribute to the severity of the disease (Grgic *et al*., 2009). Natural co-infection with 4/91 relevant isolate of IB virus and H9N2 non-highly pathogenic avian influenza virus has frequently occurred in Iranian broiler chicken farms experiencing high mortality (Seifi *et al*., 2010).

Our results showed a significant difference in weight gain between unvaccinated and vaccinated challenged groups. This may be explained as the affected chicks suffered from depression and marked reduction in feed consumption which results in significant loss of body weight from day 3 post infection as reported in study of Otsuki *et al*. (1990). The economic impact of IB infection, including poor weight gain in broilers and egg production decline in layers is well documented in the literature (Ignjatovic & Sapats, 2000; Grgic *et al*., 2009). The chicks in the unvaccinated challenged group showed a significantly lower weight gain in comparison with vaccinated challenged groups.

All groups of chicks showed lower antibody titre than the chicks of unvaccinated challenged group at the age of 13 days; one day before booster dose vaccination. It is believed that maternally derived antibodies interfere with IB vaccines administered on 1 day-olds (Mondal & Nagi, 2001; Cardoso *et al*., 2006). Vaccinated challenged groups had a higher titre than unvaccinated challenged at the age of 35 days as also reported by Thompson *et al*. (1997). At the end of the experiment, the chicks in group 5 had higher antibody titres than those of the chicks of groups 2 and 4. This result was not exactly same as the study of Terregino *et al*. (2008) who found the highest titres in the birds that received homologous antigens to the IBV vaccine strains. However, it is possible that some IBV strains have antigens in common with other IBV types (Gelb *et al*., 1981). On the other hand, many studies have shown that the situation of humoral antibody against IBV does not exactly correlate with the protection; that is, vaccinated chickens may be protected against IBV respiratory disease irrespective of the titre of serum antibody, in this case the protection is attributed to mucosal immunity (Raggi & Lee, 1965; Ignjatovic & Galli, 1994).

The infectious bronchitis virus replicates in ciliated epithelium cells of trachea and cause ciliostasis. The ciliostasis test is used to determine the damage to the trachea following growth of IBV in this tissue. The protection percentage of the cilia in groups 2 and 4 which received at least one homologous vaccine was significantly higher than in birds vaccinated with the heterologous group. Although the protection with homologous strain was indicated in many studies (Jackwood *et al*., 2003; Lin *et al*., 2005; Lee *et al*., 2010), sufficient protection was also induced in the chicks of group 5 that received heterologous vaccines. Heterologous protection, however, is much more in doubt, but there is evidence that CMI responses occur following vaccination with the H strain (Timms & Bracewell, 1981). Furthermore, it has been suggested that the CMI response to H120 vaccination may be cross-reactive (Raj & Jones, 1997). The H strain, a Massachusetts IB vaccine serotype, has shown a rare ability to cross-protect against heterologous serotypes, thus making it potentially effective for controlling a broader range of serotypes (Bijlenga *et al*., 2004). The H120 vaccine was found to induce some protection of the respiratory tract against heterologous challenge with strains of the Belgian B1648, French 84084 and French 84221 serotypes (Cook *et al*., 1999). Moreover,

it is likely that the N protein contribute to cross immunity since this protein of the different IBV isolates differed by only about 5% among IBV isolates (Williams *et al*., 1992). Beaudette vaccine strain used for *in ovo* vaccination on 18-day-old embryos caused a peak of ciliostasis 5 days post hatch (Tarpev *et al*., 2006). In this experiment the peak of ciliostasis was found 2 and 3 days post challenge and the ciliostasis levels decreased after this time (data not shown).

The results of ciliostasis test and detection of challenged virus in different samples had a similar pattern, indicating the protection as shown previously (Marquardt *et al*., 1982). Declaring a chicken as non-protected due to the recovery of small amounts of challenge virus in the presence of normal ciliary activity is too stringent (Cavanagh, 2003). Although the blood antibodies are not very effective against IBV infections, high levels of antibody in the blood have the potential to 'leak' into the respiratory tract mucosa and thereby provide protection (Jackwood, 2010), so it may be the case of chicks in group 5.

The clinical signs combined with the results of gross pathology, weighing and cilia activity showed the clinical safety of the two commercial vaccines used. Post vaccinal reaction observed in the field following IB vaccination could be attributed to secondary bacterial infection and husbandry practices (Cook *et al*., 1986; Matthijs *et al*., 2003).

The maximum number of positive challenge viral genome was in the trachea 2 and 4 days PI. The data resembled reports from previous studies (Hofstad & Yoder, 1966; Ambali & Jones, 1990). The challenging virus was not detected in tracheas of groups 2 and 4, four days post challenge, indicating sufficient protection with homologous vaccines. In addition, birds of group 5 also provided good protection against the challenging virus. The H120 and 4.91 vaccinal viruses were not detected 17 days post vaccination in groups 1 and 6. Alvarado *et al.* (2006) showed that after vaccination of mass strain at 1 day of age by coarse spray, virus was detected by reverse transcriptase–polymerase chain reaction up to 14 days that was in agreement with this study.

The data presented here showed that a Massachusetts serotype vaccine like the H120 vaccine provides, to some extent, protection against the field isolate, but using a heterologous vaccine induced better protection. Since more than one serotype persists in the field (Seyfiabad Shapouri *et al*., 2004; Ahmed *et al*., 2007; Roussan *et al*., 2008), the use of different serotype vaccines may produce better efficacy. The results also showed that all vaccination programmes achieved considerable protection against the challenge virus; however, the vaccination failure sometimes occurs in the field, probably due to the existence of other IBV variants or substandard management practices.

ACKNOWLEDGEMENT

This research financially supported by a grant of the Shiraz University Research Council.

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Paper received 25.06.2012; accepted for publication 27.09.2012

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