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Evaluation of protection conferred by a vaccination program based on the H120 and CR88 commercial vaccines against a field variant of avian infectious bronchitis virus

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Primary Audience: Veterinarians, Researchers, Flock Supervisors

SUMMARY

Due to serotype variations among different avian infectious bronchitis viruses isolated in Tunisia since 2000, protection of chicks, especially broiler flocks, with Mass H120 vaccine often fails. Therefore, association of CR88 (793B type) with H120 vaccines was used for better response. Challenge experiments were then conducted to evaluate tracheal and renal cross-protection in chickens immunized via nasal and eye drops. Conferred protection was measured by clinical signs and macroscopic lesions observed, based on scores attributed according to their severities. The results showed a low protection conferred by H120 alone, as vaccination did not reduce tracheal and kidney lesions (70% scored as 3) after TN20/00 virus challenge, which also led to 10% mortality. Conversely, the challenge results indicated that the combination of the 2 strains (H120/CR88) allow high protection. Based on the results of the challenge experiments, a vaccination protocol coupling CR88 to H120 was applied for industrial broiler flocks. Clinical observations and serological results confirmed that association of heterologous serotypes (H120 and CR88 vaccines) increased the levels of protection against infectious bronchitis viruses compared with the H120 vaccine given alone.

Key words: infectious bronchitis virus, vaccination, cross-protection, challenge, antibody titer, field variant isolate, MassH120 vaccine, CR88 variant vaccine

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DESCRIPTION OF PROBLEM

Coronaviruses infect a wide range of animals and cause respiratory, enteric, hepatic, and neurological diseases of varying severity [1]. They cause severe diseases in livestock animals and thereby lead to high economic losses [2]. Based on genotypic and serological characterization, coronaviruses are divided into 4 distinct groups. Their tendency for recombination and high mutation rates may allow their adaptation to new hosts and ecological niches [3–6]. Infectious bronchitis virus (IBV), the coronavirus of chickens, is one of the foremost causes of economic losses for the poultry industry, affecting the performance of meat and egg-laying birds,

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mainly through respiratory signs and severe egg drop and poor quality, respectively [7]; kidney damage can also occur [4, 8]. Infectious bronchitis virus has the ability to change rapidly [9]. In Tunisia, where the poultry industry is of great importance socially and economically, IBV remains one of the main causes of infectious disease-related economic losses, although vaccination programs based on live H120 strain vaccine are being used [10]. Despite the development of vaccines that contribute to the control of the clinical disease, the emergence of novel infectious bronchitis (IB) variant viruses make it difficult to control it, as no cross-protection exists between various IB strains [4].

To understand epidemiological conditions and virus changes, efforts have been undertaken since 2000 to isolate and type IBV field strains. Modern intensive poultry production practices in Tunisia have contributed to the presence of new IBV variants, which can evade the immune response induced by the commercial H120 vaccine, the only vaccine in use in the country [10], as multiple IBV serotypes are recognized worldwide [11, 12]. Previous studies in Tunisia indicated that these field IBV variants arise from recombination, insertions, deletions, and point mutation events, especially in the S1 gene (spike glycoprotein gene) resulting in the generation of 5 Tunisian IBV variants TN20/00, TN200/01, TN335/01, TN296/07, and TN556/07 [13, 14]. Pathogenicity testing was important and showed that these field isolates produced typical lesions in the respiratory tract and severe damages in the kidney of infected birds. The TN20/00 variant was identified as a nephropathogenic IBV, not related to the Massachusetts serotype and showing higher pathogenicity [10]. Serological and molecular studies confirmed that the Tunisian IB variant viruses isolated from vaccinated chicken flocks are genetically different from the vaccine in use [14]. In a sero-surveillance survey in vaccinated flocks, low levels of antibodies were found, indicating vaccination failures; 45 to 69% of the flocks showed positive levels of antibodies and only 5 to 15% of these were protected [10]. In general, vaccination fails and incidence of IB outbreaks are highly associated to the vaccination process of each country, independent of the virus strain [15]. In fact, the type of virus strain infecting a flock determines the pathogenesis of the disease and the outcome of the infection is closely dependent on the immune status of the host [4, 16, 17].

The present study was designed to determine if cross-protection against the Tunisian TN20/00 virulent field isolate is induced by the commercially available H120 vaccine and to evaluate a vaccination protocol coupling the 2 vaccine strains H120 and CR88 in a challenge experiment using the TN20/00 variant isolate. Likewise, the levels of respiratory tract and kidney protection were studied in flocks vaccinated either with the H120 serotype alone or combined with the CR88 strain.

**MATERIALS AND METHODS**

The current study included 2 distinct trials involving both laboratory and field experiments. The first study was a vaccine challenge study against IBV carried out in vivo on 70 conventional broiler chicks divided into 7 different groups. The program of the vaccination was based on the combination of the 2 different vaccines strains, H120 and CR88, and was evaluated by the protection level conferred against an experimental challenge using a local IBV variant strain. The second study was a field study conducted on commercial broiler chicken flocks. A vaccination schedule included the CR88 vaccine with the H120 strain and was applied on 4 different flocks, where 1 flock was vaccinated only with the H120 strain as a control flock.

**Experimental Study**

**Experimental Chickens.** Seventy 1-d-old conventional broiler chicks were obtained from a local hatchery [18]. Chickens were housed in isolators [19] from d 1 until the end of the experiments.

**Virus Strains.** Two different live vaccines were used: the commercial Massachusetts vaccine, H120 strain [20], and the commercial CR88 vaccine, 793B type [21]. The vaccines were used as recommended by the manufacturers. The challenge IBV TN20/00 strain (Laboratory of Epidemiology and Veterinary Microbiology, Institute Pasteur Tunis, Tunis, Tunisia; Gene Bank accession number: EF535998), a Tunisian pathogenic variant, was isolated at
the Laboratory of Epidemiology and Veterinary Microbiology, Institute Pasteur Tunis, in 2000 from broilers with nephritis (from flock located in Tunis). The TN20/00 strain is serologically and genetically very distant from the Massachusetts strain, and the S1 glycoprotein gene sequence comparisons showed significant differences and major changes, mainly in nucleotide and amino acid sequences of hypervariable regions of TN20/00 compared with the H120 strain (50% nucleotide and 64% amino acid similarities) [13]. The observed variation in the hypervariable region sequences was associated with changes in S1 antigenic sites sequences. The virus was kept at −80°C, as a virus-containing allantoic fluid.

**Virus Titration.** Virus titrations of vaccine and challenge viruses were performed in 10-d-old embryonated specific-pathogen-free White Leghorn eggs following the method of Reed and Muench [22]. The Massachusetts H120 and the CR88 vaccines had titers of 10⁴.⁷⁵ and 10³ 50% embryo infectious dose (EID₉₀) per milliliter, respectively. The IBV Tunisian TN20/00 isolate had a titer of 10⁵ EID₉₀/mL.

**Experimental Design.** At d 1, chickens were individually tagged and weighed, then divided into 7 treatment groups with 10 birds per group. Each group was kept in a separate isolator during the 40-d experiment. The birds from groups 1, 2, 3, and 5 were vaccinated via eye drops with 1 dose of 10³ EID₉₀/mL per bird of H120 vaccine (0.2 mL) at 1 d of age. At 14 d of age, birds from group 2 and birds from groups 1, 4, and 5 received 1 dose per bird of H120 and CR88 vaccines via eye drops. At 28 d of age, birds of groups 1, 2, 3, 4, and 6 were challenged by eye-nose drops with 1 dose of 10⁵ EID₉₀/mL of challenge virus TN20/00 variant (0.05 mL in each eye and nostril). Groups 5 and 6 were used as a vaccinated (vaccinated-unchallenged) and challenged controls (unvaccinated-challenged), respectively. The seventh group was left as a negative control (unvaccinated-unchallenged; Table 1).

**Bird Care.** The bird care and experimental protocol were approved by the ethical committee on medical and animal research of the Institute Pasteur de Tunis.

**BW.** Birds were weighed individually at d 1 (uniformity of the groups at the start of experiment), at d 28 (challenge day), and at the end of the experiment. Average weight gain (WG) was calculated for each group between d 28 and 40.

**Clinical Observations and Postmortem Lesions.** Clinical signs resulting from IBV infection were registered daily, between 28 and 40 d of age, according to their severity as 0 = healthy, no clinical signs; 1 = depression; 2 = sick, coughing, sneezing, nasal discharge; and 3 = severe respiratory distress, death. Mortality was recorded daily and dead birds were necropsied to determine the cause of death.

**Serological Responses.** At d 1, 7, 14, 28, and 40, blood samples were collected from all bird groups. The sera were used to measure the level of antibody (Ab) to IBV for each surviving bird by ELISA and expressed as antilog of log₁₀ titer according to the manufacturer instructions [23].

**Postmortem Examinations.** At the end of the experiments, birds were euthanized and macroscopic lesions observed in trachea, lungs, and kidney were registered according to predefined scores as 0 = no lesions; 1 = serous exudates in the trachea, petechial hemorrhages; 2 = inflammation, severe congestion of lungs, pneumonia; and 3 = kidney damage, severe renal hemorrhages, nephritis, swollen, pale kidney, with distended tubules, and ureters containing urate crystals.

**Statistical Analysis.** Data were analyzed using Fisher’s exact test for comparisons and to obtain a statistical analysis of differences between groups at a 5% significance level.

**Field Study**

**Chickens Flocks: Source and Sampling.** This study was carried out on commercial broilers...

| Group | Birds | Age (d) | Vaccination Schedule |
|-------|-------|---------|----------------------|
| 1     | 10    | 1       | Mass H120 CR88       |
| 2     | 10    | 14      | Mass H120 Mass H120  |
| 3     | 10    | 14      | Mass H120            |
| 4     | 10    | 14      | — CR88               |
| 5     | 10    | 14      | Mass H120 CR88       |
| 6     | 10    | 14      | — —                  |
| 7     | 10    | 1        | Unvaccinated-unchallenged |

¹Birds were given the challenge virus at d 28.
er chickens provide from commercial flocks located in the north of Tunisia (Centre Chaffrou, Jedaida, Tunis, Tunisia). A total of 51,770 one-day-old commercial broiler chickens were included in this study. The flocks were reared under strict hygienic conditions in 4 separate buildings (B1 to B4). Broilers in house B1 were vaccinated at 1 and 10 d of age with H120 vaccine and were kept as a control flock. Chickens housed in buildings B2, B3, and B4 were vaccinated at 1 and 10 d of age with H120 and CR88 vaccines, respectively.

**Serological Analysis.** Blood samples were collected from all the flocks (20 blood samples per building) at 1, 10, and 38 d of age and the day of slaughter. Antibodies against IBV were determined using an ELISA kit [23], following the manufacturer’s recommendations, and mean titers were calculated. For statistical analysis, the geometric mean titer (GMT) values were calculated by the FlockChek IBV software and the mean titers evolution of the control and assay flocks were calculated using the Wilcoxon 2 tailed test.

**BW, Clinical Observations, and Mortality.** Chickens were weighed and WG were calculated. Clinical signs resulting from IB vaccination were determined and mortality recorded daily. Cause of chickens’ death was determined by postmortem examination.

**RESULTS AND DISCUSSION**

Production of new commercial vaccines against new IBV variants is generally not beneficial to the concerned biological firms because of the high expenses and the period required for their product validation [16]; besides, the relationship between protection and serotype is often hard to evaluate for the choice of the right vaccine serotype [24, 25]. For these reasons, assessing antigenic relationships among field and vaccine IBV strains must be accomplished by standard methods that mainly include laboratory cross-challenge studies in chickens. In the current study, protection conferred by the H120 vaccine (Massachusetts group) against the Tunisian TN20/00 variant was evaluated. In addition, the protection offered by the vaccine was compared with the protection provided by the CR88 variant vaccine (793B type) alone or in association with the H120 vaccine, under laboratory conditions. In addition, a field study was carried out to evaluate the efficacy of a vaccination protocol based on the administration of H120 vaccine at 1 d followed by a second vaccination with the CR88 strain at 14 d old. The antigenic relatedness between the CR88 and the TN20/00 serotypes in addition to their renal tropism were the main factors for using the CR88 vaccine strain in the current study. Bourogaâ et al. [14] demonstrated by phylogenetic studies that the CR88 vaccine was closely related to the Tunisian field variants, especially the TN20/00 variant.

**Experimental Study**

**Serological and Clinical Results.** Serological results using an ELISA test in the challenge assay indicated that all the birds had an Ab level of 2,084 GMT at day of hatch corresponding to the maternally derived Ab. At 28 d of age, Ab titers were low in all groups (Table 2). For all vaccinated groups, the Ab titers detected after challenge at 40 d of age of surviving birds ranged between 367 and 1,401. Group 2, double vaccinated with H120 at 1 and 14 d of age, showed the highest Ab level (1,401), which was generally expected because we used the same vaccine strain as booster. Very low ELISA titers were seen for the control groups 6 and 7 (unvaccinated-challenged and unvaccinated-unchallenged, respectively). The ELISA results indicated that the titers at 40 d of age are very low and similar among challenged and unchallenged chickens (except birds in group 2); this result suggests that serum Ab titers do not always correlate well with the presence or the lack of homologous or heterologous protection, because local Ab responses and cell-mediated immunity play a significant role in the total protection against IBV infections, as mentioned by other researchers [25–27].

Body weight and clinical observations results were different among tested groups. Average WG between 28 and 40 d of age was higher in the control unchallenged groups (5 and 7) than in the challenged groups (Table 3), averaging 208.0 and 121.0 g, respectively. However, WG did not differ significantly ($P = 0.75$) between vaccinated and unvaccinated groups (1, 4, and
Clinical signs were observed between 5 and 8 d postchallenge. All the birds in the control groups (5 and 7) showed clinical protection with no specific signs observed (Table 3), whereas challenged birds showed different rates of clinical signs. Clinical scores of sick birds in vaccinated challenged groups were between 1 and 3, and, as a consequence, 10, 30, 60, and 70% of vaccinated chickens in groups 1, 2, 3, and 4, respectively, were not totally protected against the eye-drop challenge with the TN20/00 variant virus. However, when the CR88 vaccine strain was combined with the H120 vaccine and administered at 14 d of age (Group 1), 90% of birds were totally protected and did not present clinical signs. Statistical analysis revealed significant differences ($P < 0.05$) between group 1 and groups 3 and 4 vaccinated only with the H120 and CR88 vaccines, respectively.

**Mortality.** Mortality rates recorded after challenge with TN20/00 variant are summarized in (Table 3). No mortality was observed in the control groups 5 (vaccinated-unchallenged) and 7 (unvaccinated-unchallenged). The mortality in the vaccinated-challenged groups occurred between 7 and 10 d postchallenge, and no significant difference was revealed. Among the challenged groups, no mortality was observed in group 1 (vaccinated with H120 and CR88) and percentages of mortality were 10% in group 2 (double vaccinated with H120), 10% in group 3 (vaccinated with H120), and 20% in group 4 (vaccinated with CR88). The unvaccinated-challenged chicken (group 6) showed 50% mortality. Comparison of mortality between vaccinated-challenged group (group 1) and unvaccinated-challenged group (group 6) showed significant differences ($P < 0.05$). The TN20/00 challenge induced high mortality and a decrease in growth in challenged birds, indicating that the challenge virus was highly pathogenic for the level of immunity induced by the vaccines, as reported by Yunis et al. [28]. Conversely, the mortality and the severity of the lesions were influenced not only by the challenge strain, but also by the age of the chicken and the route of vaccination [15, 17, 29–32].

**Postmortem Examination and Lesion Scores.** Lesion scores ranged between 2 for lung congestion and 3 for either severe renal hemorrhages or nephritis with swollen and pale kidney and ureters containing urate crystals; this was observed in both surviving and dead birds of all the groups (Table 3), with the exception of the group 7 (negative control group), which showed birds without macroscopic lesions. Challenged-vaccinated or -unvaccinated groups comprised 20 to 40% of the birds with a score of 2, indicating severe congestion of the lungs, liver, and spleen, and 20 to 80% with a score of 3, indicating renal lesions. Group 1 (H120 and CR88 vaccinated-challenged) showed a lower percentage of chickens with kidney damages than the group 2 (H120 double vaccinated-challenged), scored as 3 for 20 and 60% of the birds, respectively. The difference between groups 1 and 2 was significant ($P < 0.05$). Groups 3 (H120 vaccinated-challenged) and 4 (CR88 vaccinated-challenged) presented lesions scored as 3 for 70 and 60% of the birds, respectively. The analysis of postmortem results confirmed significant differences between group 1 and groups 2, 3, 4, and 6 ($P < 0.05$). When the TN20/00 variant was used to challenge birds, vaccinated chickens in group 1 were protected, whereas chickens in group 2, 3, and 4 were not protected. In fact, based on the high percentage of mortality and trachea, lung, and kidney damages, the birds vaccinated with only the H120 vaccine (group 2) presented 70% of lesions scored 3, which we interpreted as a lack of cross-protection between H120 and

Table 2. Infectious bronchitis virus geometric mean antibody titers (GMT) detected by ELISA test; presented as GMT$^1$ (CV$^2$)

| Group $^3$ | Prechallenge | Postchallenge |
|-----------|--------------|--------------|
|           | d 21         | d 28         | d 40         |
| 1         | 308 (42.12)  | 77 (49.53)   | 495 (69.54)  |
| 2         | 308 (33.6)   | 64 (50.25)   | 1,401 (39.9) |
| 3         | 140 (37.82)  | 139 (60.39)  | 209 (66.49)  |
| 4         | 300 (42.73)  | 161 (62.95)  | 367 (69.5)   |
| 5         | 308 (43.89)  | 71 (79.2)    | 668 (83.01)  |
| 6         | 69 (29.1)    | 55 (32.41)   | 24 (40.57)   |
| 7         | 69 (30.01)   | 55 (31.38)   | 27 (45.2)    |

$^1$Antibody titer = antilog of log$_{10}$ titer according to the Idexx-ELISA kit [23].

$^2$The ELISA-deduced CV percentages.

$^3$Group 1 received H120 on d 1, CR88 on d 14, and TN20/00 on d 28; group 2 received H120 on d 1 and 14 and TN20/00 on d 28; group 3 received H120 on d 1 and TN20/00 on d 28; group 4 received CR88 on d 14 and TN20/00 on d 28; group 5 received H120 on d 1 and CR88 on d 14; group 6 received TN20/00 on d 28; group 7 was the control group.
The results indicated good protection against challenge with TN20/00 when the CR88 variant vaccine was administered at 14 d of age to chickens vaccinated with H120 at d 1 (group 1). Low clinical signs and lesion scores were observed in birds from group 1 as compared with those of groups 2, 3, 4, and 6 ($P < 0.05$). However, the presence of severe damages in birds double vaccinated with H120 (group 2), with no significant difference compared with that afforded by H120 vaccination at d 1 (group 3), denotes a lack of complete protection in these groups; however, improvement in the level of protection was noted when comparing these findings to those observed in birds vaccinated with only CR88 at 14 d old (group 4). The renal lesions observed in dead birds of groups 4 and 6 (60 and 80% scored as 3, respectively) were expected because of the renal tropism of the coronaviruses CR88 and TN20/00, in addition to their respiratory tropism described in previous studies [10, 33].

### Table 3. Clinical and postmortem examination results$^{1,2}$ of the challenge experiments

| Group$^3$ | Mean weight gain (g) | Mortality (%) | Clinical sign | Macroscopic lesion |
|-----------|----------------------|---------------|---------------|--------------------|
|           |                      |               | %             | %                  |
|           |                      |               | Score$^4$     | Score$^5$          |
| 1         | 100                  | 0$^a$         | 90$^a$        | 80$^{AB}$          |
|           |                      |               | 10$^a$        | 20$^{AB}$          |
|           |                      |               | 70            | 40$^{AB}$          |
|           |                      |               | 20 1–2        | 60$^{AB}$          |
|           |                      |               | 10            | 60$^{AB}$          |
| 2         | ND$^d$               | 10            | 40$^a$        | 30$^{AB}$          |
|           |                      |               | 50$^a$        | 70$^{AB}$          |
|           |                      |               | 10            | 3                  |
| 3         | ND                   | 10            | 30$^a$        | 40$^{AB}$          |
|           |                      |               | 50$^a$        | 60$^{AB}$          |
|           |                      |               | 20$^a$        | 3                  |
| 4         | 115                  | 20            | 30$^a$        | 1                  |
|           |                      |               | 50$^a$        | 3                  |
|           |                      |               | 20$^a$        | 3                  |
| 5         | 372                  | 0             | 100           | 80                 |
|           |                      |               | 20            | 2                  |
| 6         | 150                  | 50$^{AB}$     | 40            | 80$^{AB}$          |
|           |                      |               | 10            | 20$^b$             |
|           |                      |               | 50            | 3                  |
| 7         | 244                  | 0             | 100           | 100                |

$^a$Capital letters represent significant differences between clinical signs, mortality, and lesions of the experimental groups at $P < 0.05$.

$^1$Results are corresponding to mean weight gain between 28 and 40 d of age, mortality percentages, and clinical sign and macroscopic lesion scores for trachea, lung, and kidney tissues following challenge with TN20/00 isolate.

$^2$Parameters were assessed starting on d 5 postchallenge until 40 d of age in dead and surviving chickens.

$^3$Group 1 received H120 on d 1, CR88 on d 14, and TN20/00 on d 28; group 2 received H120 on d 1 and 14 and TN20/00 on d 28; group 3 received H120 on d 1 and TN20/00 on d 28; group 4 received CR88 on d 14 and TN20/00 on d 28; group 5 received H120 on d 1 and CR88 on d 14; group 6 received TN20/00 on d 28; and group 7 was the control group.

$^4$0 = healthy, no clinical signs; 1 = depression; 2 = sick, coughing, sneezing, nasal discharge; 3 = severe respiratory distress, death.

$^5$0 = no lesions; 1 = serous exudates in the trachea, petechial hemorrhage; 2 = inflammation, severe congestion of lungs, pneumonia; 3 = kidney damages, severe renal hemorrhages, nephritis, swollen, pale kidney, with distended tubules, and ureters containing urate crystals.

$^d$ND = not done.
that S1 protein is responsible for the protective effect of vaccination with 2 different IBV vaccines against viral challenge in chickens.

**Field Study**

General performances and WG of the tested flocks on one side and the level of postvaccination Ab production on the other side showed that association of H120 with CR88 ameliorated bird immunity level. In fact, the resulting cross-protection induced higher levels of Ab associated with increased WG (71 g of BW in the assay flocks, B2, B3, and B4, compared with the control flock, B1) and good performance during the fattening period, including (1) no severe clinical signs in all the tested flocks throughout the study, (2) a decreased average of mortality, from 6 to 4.5%, between control B1 receiving only H120 vaccine and tested flocks (B2, B3, and B4) receiving both vaccines (H120 and CR88), respectively (Table 4). The levels of Ab against IBV in each flock (20 samples per flock), presented as GMT (Table 5), indicated that all chickens had maternally derived Ab against IBV H120 at 1 d old (GMT titers ranged from 4,559 to 7,412). At 10 d of age, birds in all flocks showed decreasing levels of Ab. At 38 d of age the control flock (B1) showed 1,067 GMT titer, whereas birds vaccinated with H120 and boosted with CR88 (B2, B3, and B4) showed Ab levels ranging from 1,870 to 4,028 (average GMT titer was 2,692 for these flocks). Consequently, chickens receiving both vaccines produced higher Ab titers than birds of the control group. However, the variation between flocks receiving the same treatment indicated the heterogeneity of the immune response among birds of each flock (CV >50%). Based on the results of the present study and the challenge experiment results reported by others [9, 16, 24, 34], an adapted program using heterologous vaccines H120 and CR88 at 1 and 10 d of age, respectively, has given better results.

**CONCLUSIONS AND APPLICATIONS**

1. The results indicated that double vaccination is more efficacious to protect chickens against TN20/00 virus infection compared with the vaccination with only 1 vaccine strain, and the protection is much better when the H120 and CR88 vaccines strains were combined. Thus, the use of H120 vaccine at 1 d of age and CR88 at 10 to 14 d of age should be recommended in Tunisian farms, but more challenge experiments testing other commercial vaccines (such IB4-91, D274, and others) may be useful to select the suitable vaccine.

2. Adapting such a program in broilers would provide an efficient protection to cover the whole fattening period and reduce economic losses caused by IBV field variant. Therefore, our results were interesting when considering the choice of adequate commercial vaccine strain for use.

3. Because the extent to which IBV infection can cause economic losses depending on the virus strain, the chicken age at

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**Table 4. Description and results**

| Item                        | Birds | Vaccine          | Age (d) | Control flock (B1) | Assay flock (B2+B3+B4) | Difference (AF – CF) |
|-----------------------------|-------|------------------|---------|-------------------|------------------------|----------------------|
| Control flock (CF)          |       |                  |         |                   |                        |                      |
| B1                          | 8,000 | MassH120         | 1 and 10|                   |                        |                      |
| Assay flock (AF)            |       |                  |         |                   |                        |                      |
| B2                          | 16,000| MassH120-CR88    | 1–10    |                   |                        |                      |
| B3                          | 14,190| MassH120-CR88    | 1–10    |                   |                        |                      |
| B4                          | 13,400| MassH120-CR88    | 1–10    |                   |                        |                      |

Comparative performance of vaccinated flock

| Item                        |                   |                   |       |
|-----------------------------|-------------------|-------------------|
| Mean BW (kg)                | 1.650             | 1.721             | 0.71  |
| Consumption index           | 1.910             | 1.883             | −0.027|
| Mortality (%)               | 6                 | 4.5               | −1.5  |

1Results recorded after vaccination with H120 alone or associated to CR88 considering BW and mortality.
infection, nutrition, and environmental conditions within the poultry flock, biosafety is likely to be insufficient, as the virus spreads rapidly. Consequently, the identification of newly introduced IBV serotypes or variant strains can be useful to modify prophylactic programs providing greater protection against enzootic strains, and the vaccination must be commonly practiced within the adapted program to the field needs.

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