Protection of chickens vaccinated with different schemes including the 4/91 IBV vaccine strain against field IBV strain Italy 02: preliminary results

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ABSTRACT

The ability of different vaccine programmes (including the 4/91 vaccine strain) to protect against field infectious bronchitis virus (IBV) strain Italy 02 was investigated using specific pathogen free (SPF) chickens. Protection, as measured by assessing ciliary activity of the tracheal epithelium following challenge, was excellent with all vaccine schedule used in this trial. The data provided by this study also indicates that vaccination programmes induced adequate protection against both challenges at 36 and at 56 days of age.

Key Words: Infectious bronchitis virus (IBV), Vaccination, Specific pathogen free (SPF) chickens, Italy 02 IBV strain.

RIASSUNTO

PROTEZIONE DI POLLI VACCINATI CON DIFFERENTI PROGRAMMI VACCINALI NEI CONFRONTI DEL CEppo DI CAMPO IT-02 DEL VIRUS DELLA BRONCHITE INFETTIVA: RISULTATI PRELIMINARI.

E’ stata condotta una prova sperimentale al fine di valutare la protezione indotta da differenti programmi vaccinali (comprendenti anche il ceppo vaccinale 4/91) nei confronti dell’infezione sostenuta dal ceppo di bronchite infettiva aviaria denominato Italy 02. Il livello di protezione è stato calcolato attraverso la valutazione della ciliostasi osservata su colture d’organo (anelli tracheali). Sono stati rilevati buoni indici di protezione in tutti i programmi vaccinali impiegati nella prova. Inoltre, tale protezione è stata osservata in entrambe le età utilizzate per il challenge con il virus di campo (36 e 56 giorni).

Parole chiave: Virus della bronchite infettiva aviaria (IBV), Vaccinazione, Polli SPF, Ceppo It-02 dell’IBV.

Introduction

1999. An IBV strain isolated in Italy for the first time at the IZSLER laboratory in Forlì on SPF eggs from a broiler flock with respiratory disease. The strain was named 4682/FO.

2000. The cooperation work between the lab IZSLER in Forlì and “All Russian Research Institute for Animal Health (ARRIAH) – Russia” starts. Dr. Vladimir Drygin made the sequence analysis on 10 IBV strains isolated from IB outbreaks in Italy by the IZSLER Forlì lab during the year 1999. He renamed all the IBV tested strains from It-01 to It-10. The IBV strains It-02, IT-04 and It-08 were found to be genetically different from the other strains tested.

2002. It-02 sequence published on NCBI-BLAST (accession number AJ457137). 2004. It-02 is widespread in Europe. It is the predominant genotype in the UK, but also in Germany, France, Spain, The Netherlands it is causing problems (Worthington et al., 2004).

The aim of the present study is to investigate the efficacy of different IB vaccine programs against an It-02 challenge in SPF chickens reared in isolators.
Material and methods

Experimental design: the experimental design is presented in Table 1. Three groups (A, B, C) of 20 specific pathogen free (SPF) chicks, 1 day old and housed in separate negative pressure isolators, were vaccinated by the oculonasal route (o.n.) with $10^3$ EID$_{50}$ of the Nobilis IB Ma5 vaccine. At 1 day old, the group B was vaccinated with $103.6$ EID$_{50}$ of Nobilis IB 4/91 too. At 2 weeks of age, the groups A and C were vaccinated o.n. with $10^3.6$ EID$_{50}$ of the Nobilis IB 4/91 vaccine. Two unvaccinated groups (D, E) were taken as control.

At 5 weeks of age, the control group D that had received no vaccinations and groups A and B vaccinated with Ma5 and 4/91, received a very strong challenge by oculonasale route with $10^7.5$ EID$_{50}$ of the Italian isolate It-02 strain (4682/FO) assayed in tracheal organ cultures. The fourth vaccinated group C was challenged with the same strain at the same concentration at 56 days of age. The fifth group E, served as the not vaccinated unchallenged control group.

At 4 and 7 days post-challenge, 4 chicks of each group was suppressed. The tracheas were removed and examined for ciliary activity as reported by Cavanagh et al. (1997). Each one of 10 tracheal rings prepared from each trachea was examined by low-power microscopy and ciliary activity scored as follows (Cook et al., 1999): 0 - all cilia beating; 1 - 75% beating; 2 - 50% beating; 3 - 25% beating; 4 0% beating (100% ciliostasis). This gives a maximum ciliostasis score of 40 for each trachea. A chick is intended as protected if the ciliostasis score for trachea is less than 20. For each group, the protection score was calculated according to the following formula:

$$\left(1 - \frac{\text{mean ciliostasis score for vaccinated/infected group}}{\text{mean ciliostasis score for challenged controls}}\right) \times 100$$

The higher the score, the better the level of protection provided by the vaccination programme.

Laboratory investigations: in all groups the serology at the 2nd, at the 4th and at the 9th week of age has been assessed testing sera for IB antibodies by Hemagglutination-inhibition (HI) and ELISA tests. 20 chicks per group were tested for HI using a M41Antigen and 793/B antigen (made by Central Veterinary Laboratory – Weybridge UK) and an Italy 02 antigen (made by IZSLER Brescia). The Elisa test has been performed with a commercial ELISA test (Synbiotics®). IBV isolation have been performed on all groups 7 days after challenge as in Table 2 using SPF eggs inoculated via the allantoic sac. Reverse transcriptase-polymerase chain reaction (RT-PCR) and nested PCR (Cavanagh et al., 1999) have been performed on all groups 7 days after challenge as in Table 2.

Results

The results of the ciliostasis test and of the protection index are shown in Table 3 and Table 4.

![Table 1](image)
Serology

The HI test showed significant M41 titers 2 weeks after the vaccination in all groups, titers that declined 2 weeks later at 28 days, but rocketed at 62 days.

The HI test with 793/B antigen showed low antibody levels in the first two samples but a strong growth in the last one.

The HI serological data with It-02 antigen show an increase in all three sampling points (Figures 1, 2, and 3).

The Elisa test results are shown in the Fig 4. Group A showed an antibody peak at 28 days, after the revaccination with 4/91, but a decrease at 62 days. Group B showed a light increase which remained constant.

Virology

The virological tests performed were: virus isolation on SPF eggs, RT-PCR and type-specific nested PCR. The results are shown in Table 5. The virus isolation performed 7 days after the challenge was positive and the nested PCR identified the Italy 02 strain in the entire test. The isolations performed 26 days after challenge (62 days) were negative.

Discussion

Ciliostasis test

The results of the ciliostasis test show that all the vaccination schemes applied gave excellent

| Table 2. Experimental design and analysis. |
|------------------------------------------|
| Age | A | B | C | D | E |
|-----|---|---|---|---|---|
| 0   | Ma5* | Ma5+4/91* | Ma5* | - | - |
| 14  | 4/91* Serology | 4/91* Serology | Serology | Serology | Serology |
| 28  | Serology | Serology | Serology | Serology | Serology |
| 36  | It-02** Challenge | It-02** Challenge | - | It-02** Challenge | - |
| 40  | TOC | TOC | - | TOC | - |
| 43  | TOC Virology | TOC Virology | - | TOC Virology | TOC |
| 56  | - | - | It-02** Challenge | - | - |
| 60  | - | - | TOC | - | TOC |
| 62  | Serology Virology | Serology Virology | TOC Virology | Serology Virology | Serology Virology |

*IBV vaccine strains used for the vaccination
**IBV field strain used for the challenge
TOC: tracheal organ culture

Group C showed a strong increase after the challenge, as did group D.
### Table 3. Tracheal organ culture (TOC) score.

| Group               | Age of challenge | 4 days post challenge | 7 days post challenge |
|---------------------|------------------|-----------------------|-----------------------|
| A (1d Ma5+14d 4/91) | 36 days          | 2.5                   | 3                     |
| B (1d Ma5+4/91)     | 36 days          | 3                     | 4                     |
| C (1d Ma5+14d 4/91) | 56 days          | 1.5                   | 2.7                   |
| D Control infected  | 36 days          | 29.2                  | 32.7                  |
| E Control non infected | -                | 0.2                   | 0.2                   |

### Table 4. Protection Index.

| Group       | Vaccination schedule | It-02   | Protection index (4 days post challenge) | Protection index (7 days post challenge) |
|-------------|----------------------|---------|------------------------------------------|------------------------------------------|
| A           | Ma5 at 1 day 4/91 at 14 days | 36 days | 92.35                                    | 90.82                                    |
| B           | Ma5+4/91 at 1 day     | 36 days | 90.82                                    | 87.76                                    |
| C           | Ma5 at 1 day 4/91 at 14 days | 56 days | 95.41                                    | 91.74                                    |

### Table 5. Virological data.

| Group | 43 days  | 62 days  |
|-------|----------|----------|
| A     | Isolation PCR = It-02 | neg      | neg      |
| B     | Isolation PCR = It-02 | neg      | neg      |
| C     | Not done | Isolation PCR = It-02 | neg      | neg      |
| D     | Isolation PCR = It-02 | neg      | neg      |
Figure 1. HI test Group A.

Figure 2. HI test Group B.

Figure 3. HI test Group C.
VACCINATION AGAINST ITALY 02 IBV STRAIN

Protection against the challenge with Italy 02, whereas the chicks in the challenged control group were not protected.

Protection index
The results of the protection index were equally very good. These data indicate a very good protection both in the early infection at 36 days of age and in the late one at 56 days in older birds.

Serology.
HI test
The HI It-02 test shows a constant increase in all groups that could be caused by an antigenic cross reactions against both the M41 and the 793/B IBV strains. It would be interesting to investigate the real genetic relationships of the It-02 with the above-mentioned. As reported by Jackwood et al. (2005) the serotype of the IBV causing the disease must first be determined so that the birds can be properly vaccinated. The HI It-02 test show a constant increase in all groups that could mean an antigenic cross reactions against both M41 and 793/B IBV strains. Accordingly to the concept of Jakwood et al. this could explain the cross protection we proved in the present paper.

It could be interesting to investigate the real genetic relationship with these strains and with all the other main IBV strains in the world. This study is in progress.

ELISA
It is well known that the ELISA test is not specific and indicates only a general response against IB.

Group A: we had an increase after the 2 vaccinations, but a decrease after the challenge. The explanation could be that the birds were well protected at day 35 of the challenge and the virus had therefore been blocked in the trachea by a strong local immunity without the possibility to penetrate further into the organism.

Group B: we ascertained a non-significant titre increase. The explanation could be the same as that applied to group A. The birds were well protected at day 35 of the challenge and the virus had therefore been blocked in the trachea by a strong local immunity without the possibility to penetrate further into the organism. This could be due to the fact that the group received a fourfold
vaccination (1 drop of Ma5 + IB 4-91 in each eye and 1 drop of Ma5 + IB 4-91 in each nostril).

Group C: there was a strong increase, probably due to the late challenge at 56 days. Comparing these data with the TOC and the protection index results, it appears that the birds are well protected against the disease, but not against the infection. The wild virus was able to pass through the organism and to stimulate the immune system.

Virology

Both PCR and reisolation of the virus were positive for the IBV strain Italy 02. This is probably due to the very high concentration (more than 1000 times higher than the standard one fixed by the European Pharmacopoea) of the challenge virus. Nevertheless, the birds were not only well protected against the disease but, according to the different vaccination schemes, perhaps also against the infection.

This hypothesis should be confirmed through immune histochemical tests.

Conclusions

Based only on genomic data, the 4682/FO (or It-02) IBV strain is present in Italy since 1999. A correlation between genotype and serotype is hardly possible. The study is in progress.

All vaccination programs included in this laboratory trial induced protection against It-02 challenges both at 36 and 56 days of age.

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