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Evaluation of unintended 1/96 infectious bronchitis vaccine transmission in broilers after direct contact with vaccinated ones

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ABSTRACT: Infectious bronchitis virus is characterised by an extreme degree of variability which deeply affects the first-choice control strategies against the disease. Each country tends to adopt its own protocols and even vaccine producers themselves can also adopt different strategies in attempts to confront local epidemiological concerns. In the present study, we tested the potential environmental persistence, transmission ability and replication capability of a non-directly administrated vaccine strain at a hatchery and during transportation. To this purpose, we examined a single hatchery, where combined vaccination (Mass-like plus 793B-like strains) is commonly administered following the protectotype concept, whereas a single broiler flock receives only the Mass priming. Two groups of solely Mass-primed chicks were kept in contact with chicks vaccinated with both strains, during hatchery procedures and transportation, respectively. A regularly vaccinated control group was selected and all three were monitored by swab sampling until 11 days of age. Vaccine titres were quantified using vaccine-specific real-time RT-PCRs to check the kinetics of both strains. Mass titres were consistent among the groups, while the absence of the 1/96 vaccine strain in unvaccinated chicks confirmed the low risk of unintended vaccine transmission, which could complicate the diagnostic process and the epidemiological scenario.

Keywords: infectious bronchitis virus; Mass-like strain; 793B-like strain; vaccine exposure; vaccine kinetics; vaccine spreading

Infectious bronchitis (IB) was described in the USA for the first time as a respiratory disease of chickens. Its aetiological agent was isolated in 1936 and then identified as a Gammacoronavirus, namely infectious bronchitis virus (IBV) (Cook et al. 2012). Being a single-stranded RNA virus, IBV is highly susceptible to spontaneous mutation and genetic recombination, meaning that a large number of variants are circulating worldwide (de Wit et al. 2011; Jackwood 2012; Valastro et al. 2016).

IBV is extremely contagious and is easily transmitted by direct and indirect contact, due to its aerogenous spread, its high shedding titres and persistence in the environment (Cavanagh and Gelb 2008). Symptoms may vary depending on the virus strain; however, in the earliest phase, the respiratory tract is principally affected. Even though IBV is mainly a respiratory disease, depending on the strain, it can involve also the urinary tract, the gut and the reproductive system of chickens (Cavanagh and Gelb 2008). IBV is the most economically important viral respiratory disease in the poultry industry also because biosecurity alone may not be sufficient for disease control. Therefore, vaccination is widely adopted to increase the protection of chickens against

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IBV strains, to reduce the damage caused by the pathogen and to decrease the infectious pressure at the epidemiological level. Both live attenuated and inactivated vaccines have been licensed: live vaccines are used in young birds to achieve early protection and also for the priming of future layers and breeders, which are boosted by the inactivated vaccines (Cook et al. 2012). However, the use of live vaccines raises many questions about the possible circulation of the attenuated strains and the transmission of these vaccine strains from vaccinated to non-vaccinated chickens with consequent post-vaccine reactions and clinical signs of respiratory malfunction (Cook et al. 2012). A common approach is based on the combined administration of different genotypes to broaden the protection spectrum (Cook et al. 2012).

Even if the above-mentioned scenario typically applies to within-flock vaccine strain transmission, the incidental "contamination" of different chick batches during the early production phases cannot be excluded. In several hatcheries in Northern Italy, chicks are sequentially vaccinated with different vaccine strains (Franzo et al. 2016), potentially leading to unintended vaccination with undesired vaccines. In fact, many big companies or farmers often adopt different vaccination strategies, mainly with Mass priming which is sometimes administered as a single vaccination, but is more frequently followed by other strains, such as 793B-like or QX-like vaccines (Franzo et al. 2016), in line with the protectotype vaccination concept (Cook et al. 1999). Although unintended vaccination might appear harmless, it can potentially lead to problems from both clinical and epidemiological perspectives. The circulation of live attenuated strains in a partially vaccinated population can lead to rolling reactions and potentially to reversion to virulence, by allowing the repeated transmission of the vaccine virus from vaccinated birds to unvaccinated flock-mates (McKinley et al. 2008). Moreover, the widespread use of vaccines has been demonstrated to play a significant role in IBV epidemiology (Franzo et al. 2016). In particular, the involuntary spread of genotype-specific vaccines can hamper the differentiation between field and vaccine-derived strains, complicating the epidemiological scenario (Franzo et al. 2014).

Our hypothesis was that IBV live-attenuated vaccine could accidentally spread to unvaccinated groups during early production phases, affecting the epidemiological scenario. Based on these facts, the present study was aimed at exploring the potential environmental persistence, transmission ability and replication capability of a non-directly administered vaccine strain at a hatchery and during transportation.

MATERIAL AND METHODS

The experimental procedures were approved by the Institutional Review Board for Animal Research (Organismo Preposto al Benessere Animale; O.P.B.A.) at the University of Padua. The trial was carried out using Ross 308 chicks chosen from the same hatchery and breeders. The selected vaccines for the trial were based on the Mass-like strain (H120 strain; BI-VAC 1° FATRO, Italy) and 793B-like strain (1/96 strain; CEVAC IBird, CEVA, France). Full doses of both vaccines were administered by spray in accordance with the standard procedures of the hatchery. In total, 90 chicks were divided into three groups of 30 chicks each according to the administered treatment and handled in three separate and clearly labelled chick shipping boxes: control group (C): chicks vaccinated with H120 and 1/96 strains, in subsequent administrations by Desvac Duo Spray&Gel (CEVA Desvac, France). Hatchery group (H): chicks vaccinated with Mass strain alone but maintained for 5 h at the hatchery in close contact with group C. Truck group (T): chicks vaccinated with Mass strain alone but transported in a truck containing H120 and 1/96 vaccinated birds (about 53 000 chicks) including group C from the hatchery to the group C farm (4 h).

Groups H and T were moved to the Department of Animal Medicine, Production and Health (MAPS), University of Padua, Legnaro (Italy) and observed from the 1st to the 11th days of life to follow vaccine strain replication under experimental conditions. Animals were housed in two different rooms under high biosecurity conditions and examined daily for any post-vaccine reactions or

Table 1. Animal groups and adopted vaccine program

	Mass strain	1/96 strain
Control group	×	×
Hatchery group	×	–
Truck group	×	–

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clinical signs. Group C was followed over a regular productive cycle in a farm (three flocks of 17 500 birds each) where birds were confined, to compare experimental and field conditions. The used vaccination program is described in Table 1.

Samples. Ten animals from each experimental group were randomly selected for sampling and they were marked on the first day. The remaining 20 non-sampled chicks were maintained to simulate the within-flock vaccine circulation.

Samples were collected from each of the ten marked animals by taking conjunctival and choanal cleft swabs at 1, 3, 7 and 11 days of age. The swabs from each group and sampling time were pooled. Swabs were collected also from group C animals at the farm for each sampling moment. After collection, samples were transferred to the laboratory of the Department of Animal Medicine, Production and Health (MAPS), University of Padua, Legnaro (Italy). Swabs were eluted in 2 ml of PBS and then viral RNA extraction was performed using High Pure RNA Isolation kit (Roche) following the manufacturer's instructions.

After viral RNA extraction, samples were stored at −80 °C and then analysed using two different quantitative real-time RT-PCRs, specifically validated for the detection of Mass-like strains and the 1/96 strain (Tucciarone et al. 2018).

Each sample was tested using both assays to detect the presence of the 1/96 strain, due to the exposure to vaccinated animals, and to follow the kinetics of the Mass-like strain used for the vaccination of all three groups.

RESULTS

During the trial, no clinical signs were observed among the animals. Real-time RT-PCR results are reported in Table 2. Animals belonging to the control group (group C) were sampled on day 1 of the trial and they tested positive for both of the used vaccines (respectively, H120: 0.54 $\log_{10}$ infectious dose 50/ml and 1/96: 0.78 $\log_{10}$ infectious dose 50/ml) and an increasing trend in vaccine titres was observed on the following sampling times. The other two groups (group H and group T) always tested negative for the 1/96 strain but positive for the Mass strain. The Mass strain titre slightly increased over the time period under consideration in a similar way for all the groups. The kinetics of Mass vaccine viral replication for groups C, H and T is presented in Figure 1, together with the 1/96 titre trend from group C samples and negative results for groups H and T.

Table 2. Mass and 1/96 vaccine titres ($\log_{10}$ infectious dose 50/ml)

Age (day of the trial)	Control group		Hatchery group		Truck group	
	Mass	793B	Mass	793B	Mass	793B
1	0.54	0.78	0.60	neg.	0.93	neg.
3	4.08	3.36	2.98	neg.	4.78	neg.
5	4.55	3.68	5.95	neg.	5.91	neg.
7	5.02	3.99	4.82	neg.	6.08	neg.
11	5.95	4.63	5.73	neg.	5.62	neg.

DISCUSSION

The aim of this trial was the evaluation of the spreading capability of the 1/96 vaccine strain and its consequent replication immediately after vaccination, even when starting from a few copies. To this purpose, a short exposure time between vaccinated and non-vaccinated animals (i.e., at the hatchery or during the transportation) was allowed and the contamination occurrence was evaluated, mimicking the biological effect of daily working needs and conditions.

Based on the observed results (Table 1, Figure 1), the two groups kept under experimental conditions

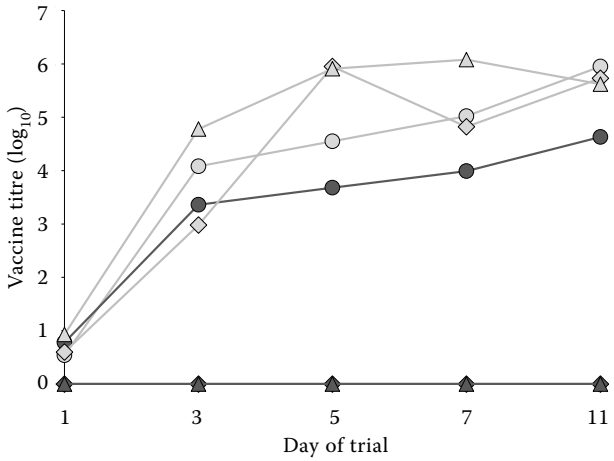

Figure 1. The kinetics of Mass and 1/96 strains for control group, hatchery group and truck group

△ = Group T Mass, ▲ = Group T 1/96, ○ = Group C Mass, ● = Group C 1/96, ◇ = Group H Mass, ◆ = Group H 1/96

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remained negative for the 1/96 strain over the whole period under consideration suggesting negligible or no spread of the studied viral strain immediately after vaccination, in spite of its well-known replication dynamics (Tucciarone et al. 2018), or the necessity of a minimum infectious dose for the vaccine to start replicating (Leyson et al. 2017). This observation can likely be explained by delayed vaccine replication, resulting in a deferred beginning of vaccine shedding and consequently the absence of spreading between the groups.

In contrast, all experimental groups (group H and group T and control group C) were positive for the Mass strain with comparable kinetics (Figure 1). This demonstrates that Mass vaccination was successful and confirms the expected Mass strain replication trends (Tucciarone et al. 2018), which can be influenced by biological factors. In fact, a negligible difference can be observed between groups H and T; similar fluctuations were also observed for other vaccine strains (Tucciarone et al. 2018). Furthermore, these results support the repeatability and safety of the vaccination strategy, which could, with confidence, be confirmed by the absence of post-vaccine reactions and clinical signs. In this study, we examined the preliminary and more practical aspects of an accidental chicken mixed vaccination/batch contamination, which can deeply obfuscate the real epidemiological situation in the field, especially considering that, for several genotypes, the routine diagnostic techniques typically do not allow a clear distinction between vaccine and field strains. The lack of replication and consequent spreading of the 1/96 vaccine strain lessens the threat of undesired vaccine circulation in population that has already received a different vaccination and suggests that the application of different vaccination protocols in the same hatchery is safe. Nevertheless, it might be the case that the working conditions and the applied practices were particularly accurate in the hatchery under investigation. In fact, it must be stressed that, independently of the vaccine features, improper vaccine handling could create a severely contaminated environment, increasing the associated risks. Other studies will be necessary to better determine the 1/96 minimum infectious dose and to evaluate the timing of replication and shedding.

In conclusion, in this study, we tackled the problem of non-direct vaccine administration using real-time RT-PCR, which allowed vaccine detection and quantification and enabled us to assess its presence and replication efficacy. A follow-up of the kinetics of the vaccine titres could help defining the conferred protection and might be useful in monitoring possible vaccine circulation, especially in cases of high-titre vaccine shedding.

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